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NEWS 21	SEP 17	CAplus coverage extended to include traditional medicine patents
NEWS 22	SEP 24	EMBASE, EMBAL, and LEMBASE reloaded with enhancements
NEWS 23	OCT 02	CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt
NEWS 24	OCT 19	BEILSTEIN updated with new compounds

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=> s multispecific ligand
L1            6 MULTISPECIFIC LIGAND

=> dup remove l1
PROCESSING COMPLETED FOR L1
L2            6 DUP REMOVE L1 (0 DUPLICATES REMOVED)

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L3            2 L2 AND PD<20020114

=> d l3 1-2 cbib abs

L3   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
1992:589579  Document No. 117:189579  Multispecific allergic reactions.
     Varga, J. M.; Klein, G. F.; Fritsch, P. (Dep. Dermatol., Univ. Innsbruck,
     Innsbruck, 6020, Austria). New Trends Allergy III, [Int. Symp.], 3rd,
     Meeting Date 1990, 100-4. Editor(s): Ring, Johannes; Przybilla, Bernhard.
     Springer: Berlin, Germany. (English) 1991. CODEN: 58CHAG.
AB   A review with 8 refs. The high prevalence of allergic cross-reactions is
     generally explained by the presence of common allergens in different
     allergen prepns., shared antigenic determinants present on different
     allergens, and/or multiple sensitizations. It is conceivable, however,
     that, in addition to these mechanisms, multispecific recognition of allergens
     by IgE antibodies may contribute to the frequently observed lack of strict
     specificity in allergic reactions. The authors have initiated a project
     to test the role of IgE multispecificity in allergic cross-reactions by
     screening monoclonal IgE antibodies for multispecific
     ligand binding. Cross-stimulation of rat basophilic leukemia
     (RBL) cells, sensitized with multispecific monoclonal IgE antibodies, is
     then analyzed. The results show that multispecific IgEs mediate
     cross-stimulation of RBL cells by chemical unrelated compds.

L3   ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
1978:20376  Document No. 88:20376  Evidence for multispecificity of antibody
     molecules. Cameron, Deborah J.; Erlanger, Bernard F. (Dep. Microbiol.,
     Columbia Univ., New York, NY, USA). Nature (London, United Kingdom),
     268(5622), 763-5 (English) 1977. CODEN: NATUAS. ISSN:
     0028-0836.
AB   Three homogeneous anti-AMP antibodies were isolated from a rabbit
```

immunized with (AMP)2-gramicidin S and tested for multispecificity by an enzyme immunoassay techniques. All 3 antibodies reacted best with AMP but they also showed crossreaction with 1, 4, and 6 other ligands resp., indicating multispecificity of the antibody combining sites. Peripheral lymphocytes from the immunized rabbit also had **multispecific ligand** receptor sites.

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=> s fusion protein
L4      221889 FUSION PROTEIN

=> s l4 and ligand
L5       18282 L4 AND LIGAND

=> s l5 and different biodistribution
L6           0 L5 AND DIFFERENT BIODISTRIBUTION

=> s l5 and biodistribution
L7          29 L5 AND BIODISTRIBUTION

=> dup remove l7
PROCESSING COMPLETED FOR L7
L8           14 DUP REMOVE L7 (15 DUPLICATES REMOVED)

=> s l8 and pd20020114
L9           0 L8 AND PD20020114

=> d l8 1-14 cbib abs

L8   ANSWER 1 OF 14     MEDLINE on STN                    DUPLICATE 1
2007274615.    PubMed ID: 17460060.   Targeted and untargeted CD137L
     fusion proteins for the immunotherapy of experimental
     solid tumors. Zhang Nan; Sadun Rebecca E; Arias Robyn S; Flanagan Meg L;
     Sachsman Suzanne M; Nien Yu-Chih; Khawli Leslie A; Hu Peisheng; Epstein
     Alan L. (Department of Pathology, Keck School of Medicine, University of
     Southern California, Los Angeles, California 90033, USA. ) Clinical cancer
     research : an official journal of the American Association for Cancer
     Research, (2007 May 1) Vol. 13, No. 9, pp. 2758-67.  Electronic
     Publication: 2007-04-25. Journal code: 9502500. ISSN: 1078-0432. Pub.
     country: United States. Language: English.
AB   Introduction: CD137L is a member of the tumor necrosis factor superfamily
     that provides a costimulatory signal to T cells.  In this study, two novel
     CD137L fusion proteins were produced and compared with
     the CD137 agonist antibody 2A.  Materials and Methods: Murine CD137L was
     linked to the COOH terminus of either the Fc fragment of immunoglobulin
     (untargeted version) or TNT-3 (targeted version), an antibody that binds
     to necrotic regions of tumors.  Groups of mice bearing established Colon
     26 tumors were then treated daily x 5 with each fusion
     protein or 2A to determine their immunotherapeutic potential.
     RESULTS: Both fusion proteins retained CD137L activity
     in vitro and TNT-3/CD137L showed tumor-binding activity by
     biodistribution analysis in tumor-bearing mice.  The
     fusion proteins also produced similar responses in vivo
     at the 1 nmol per dose range and showed a 60% (TNT-3/CD137L) or 40%
     (Fc/CD137L) survival of treated mice at 150 days after tumor implantation,
     similar to the effects of 2A.  Morphologic and immunohistochemical
     analyses showed massive central necrosis and infiltration of granzyme
     B-positive cells in necrotic areas and viable peripheral regions of
     treated tumors.  Finally, cell depletion studies showed that
     CD137L-mediated tumor regression was CD8(+) T cell dependent.
     CONCLUSIONS: From these studies, it was determined that both targeted and
     untargeted CD137L fusion proteins showed effective
     antitumor activity, but that the targeted version was more potent.
     Therefore, the use of the natural CD137 ligand is a promising
     approach to the treatment of solid tumors by virtue of its ability to
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produce physiologic costimulation within the tumor, limiting side effects often seen with agonist antibody therapies.

L8 ANSWER 2 OF 14 MEDLINE on STN
2006327896. PubMed ID: 16538504. Affibody-mediated tumour targeting of HER-2 expressing xenografts in mice. Steffen Ann-Charlott; Orlova Anna; Wikman Maria; Nilsson Fredrik Y; Stahl Stefan; Adams Gregory P; Tolmachev Vladimir; Carlsson Jorgen. (Department of Oncology, Radiology and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.. ann-charlott.steffen@bms.uu.se) . European journal of nuclear medicine and molecular imaging, (2006 Jun) Vol. 33, No. 6, pp. 631-8. Electronic Publication: 2006-03-15. Journal code: 101140988. ISSN: 1619-7070. Pub. country: Germany: Germany, Federal Republic of. Language: English.
AB PURPOSE: Targeted delivery of radionuclides for diagnostic and therapeutic applications has until recently largely been limited to receptor **ligands**, antibodies and antibody-derived molecules. Here, we present a new type of molecule, a 15-kDa bivalent affibody called (Z(HER2:4))(2), with potential for such applications. The (Z(HER2:4))(2) affibody showed high apparent affinity (K (D)=3 nM) towards the oncogene product HER-2 (also called p185/neu or c-erbB-2), which is often overexpressed in breast and ovarian cancers. The purpose of this study was to investigate the in vivo properties of the new targeting agent. METHODS: The **biodistribution** and tumour uptake of the radioiodinated (Z(HER2:4))(2) affibody was studied in nude mice carrying tumours from xenografted HER-2 overexpressing SKOV-3 cells. RESULTS: The radioiodinated (Z(HER2:4))(2) affibody was primarily excreted through the kidneys, and significant amounts of radioactivity were specifically targeted to the tumours. The blood-borne radioactivity was, at all times, mainly in the macromolecular fraction. A tumour-to-blood ratio of about 10:1 was obtained 8 h post injection, and the tumours could be easily visualised with a gamma camera at this time point. CONCLUSION: The results indicate that the (Z(HER2:4))(2) affibody is an interesting candidate for applications in nuclear medicine, such as radionuclide-based tumour imaging and therapy.

L8 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2007 ACS on STN
2005:479747 Document No. 143:6295 Multispecific antibodies specific to cell surface antigen and/or receptor for immunotherapy of autoimmune disease, transplant rejection, infection and cancer. Herman, William (Can.). Can. Pat. Appl. CA 2441653 A1 20050319, 166 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2003-2441653 20030919.
AB The invention contemplates a composition containing a multispecific **ligand** containing at least a first **ligand** binding moiety and a second **ligand** binding moiety. The first **ligand** binding moiety specifically binds with a pre-selected first affinity to at least a first **ligand.** The first **ligand** has a first **biodistribution.** The second **ligand** binding moiety specifically binds with a pre-selected affinity to at least a second **ligand.** The second **ligand** has a second **biodistribution.** The affinities of first and second **ligand** binding moieties are selected to bias the **biodistribution** of the multispecific **ligand** in favor of a selected location of one or both of the **ligands.** The first **ligand** is a cell surface marker or antigen associated with infectious or parasitic agents, diseased cells or disease-associated cells; and the second **ligand** is a CCR5, CXCR4, tyrosine kinase receptor, serine kinase receptor, Notch family receptor, decoy receptor, adhesion receptor, IL-8 receptor, CCR7, Fas receptor, etc.

L8 ANSWER 4 OF 14 MEDLINE on STN
2005381951. PubMed ID: 16023401. Design of cyclic and other templates for potent and selective peptide alpha-MSH analogues. Fung Selena; Hruby Victor J. (Department of Chemistry, University of Arizona, Tucson, 85721, USA.) Current opinion in chemical biology, (2005 Aug) Vol. 9, No. 4, pp. 352-8. Ref: 47. Journal code: 9811312. ISSN: 1367-5931. Pub. country:

England: United Kingdom. Language: English.

AB For over three decades, the design of linear peptide **ligands** often has incorporated cyclic constraints to improve potency, receptor selectivity, proteolytic stability and **biodistribution.** Its importance has been so well established that modern day schemes for **ligand**-based drug design often start with cyclization of linear peptides to rigidify peptide structure, to limit its conformational possibilities, and to find key pharmacophore elements in three-dimensional space. In the past several years, cyclic constraints have been used to develop **ligands** with improved efficacy, binding affinity, biostability and receptor selectivity for alpha-melanocyte-stimulating hormone (alpha-MSH). Furthermore, potent cyclic alpha-MSH analogues, such as MT-II and SHU-9119, have made structure-activity relationship studies and molecular modeling more useful for creating new three-dimensional, topographical pharmacophore templates.

L8 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 2

2004634940. PubMed ID: 15610604. Targeted adenovirus vectors. Mizuguchi Hiroyuki; Hayakawa Takao. (Project III, National Institute of Health Sciences, Osaka Branch, Fundamental Research Laboratories for Development of Medicine, Osaka 567-0085, Japan.. mizuguch@nihs.go.jp) . Human gene therapy, (2004 Nov) Vol. 15, No. 11, pp. 1034-44. Ref: 105. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Recombinant adenovirus (Ad) vectors continue to be the preferred vectors for gene therapy and the study of gene function because they are relatively easy to construct, can be produced at high titer, and have high transduction efficiency. However, in some applications gene transfer with Ad vectors is less efficient because the target cells lack expression of the primary receptor, coxsackievirus and adenovirus receptor (CAR). Another problem is the wide **biodistribution** of vector in tissue following in vivo gene transfer because of the relatively broad tissue expression of CAR. To overcome these limitations, various approaches have been developed to modify Ad tropism. In one approach, the capsid proteins of Ad are modified, such as with the addition of foreign **ligands** or the substitution of the fiber with other types of Ad fiber, in combination with the ablation of native tropism. In other approaches, Ad vectors are conjugated with adaptor molecules, such as antibody and **fusion protein** containing an anti-Ad single-chain antibody (scFv) or the extracellular domain of CAR with the targeting **ligands**, or chemically modified with polymers containing the targeting **ligands.** In this paper, we review advances in the development of targeted Ad vectors.

L8 ANSWER 6 OF 14 MEDLINE on STN DUPLICATE 3

2003408704. PubMed ID: 12948022. Targeting and blocking B7 costimulatory molecules on antigen-presenting cells using CTLA4Ig-conjugated liposomes: in vitro characterization and in vivo factors affecting **biodistribution.** Park Chung-Gyu; Thiex Natalie W; Lee Kyung-Mi; Szot Gregory L; Bluestone Jeffery A; Lee Kyung-Dall. (Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065, USA.) Pharmaceutical research, (2003 Aug) Vol. 20, No. 8, pp. 1239-48. Journal code: 8406521. ISSN: 0724-8741. Pub. country: United States. Language: English.

AB PURPOSE: CTLA4Ig, a **fusion protein** of CTLA-4 and Fc of immunoglobulin (Ig) heavy chain, inhibits the essential costimulatory signal for full T cell activation via blocking the interaction between CD28 and B7 molecules and renders T cell nonresponsiveness. CTLA4Ig has been used to control deleterious T cell activation in many experimental systems. We hypothesized that by conjugating CTLA4Ig to liposomes the efficacy of CTLA4Ig could be enhanced through multivalent **ligand** effect, superior targetability, and modification of the fate of ligated costimulatory molecules. METHODS AND RESULTS: Consistent with this hypothesis, liposome-conjugated CTLA4Ig bound to B7 and blocked their binding sites more efficiently than free CTLA4Ig, lowering the half maximal dose for B7 blocking by an order of the magnitude. These results

were similar both in B7-1 expressing p815 cells and in activated macrophages. Moreover, CTLA4Ig-liposomes underwent rapid internalization upon cell surface binding through B7 molecules. In allogenic mixed lymphocyte reaction assays, the CTLA4Ig-liposomes were tested to show effective inhibition of T cell proliferation. In vivo, however, when CTLA4Ig-liposomes were injected into mice, a significant fraction was localized to the reticuloendothelial system (RES), presumably because of its binding to Fc receptors expressed on tissue macrophages. The Fc receptor-mediated uptake could be alleviated by coinjection of anti-FcR monoclonal antibody. In the mouse engrafted with pancreatic islets of Langerhans underneath the capsule of one kidney, despite the increased localization in RES, enhanced accumulation of CTLA4Ig-conjugated liposome was observed in the engrafted kidney compared to the contralateral kidney. CONCLUSION: We show that the conjugation of CTLA4Ig to liposome could increase the efficiency of the targeting by increasing the binding avidity at cellular level and by increasing the concentration at the target site in in vivo system. The **biodistribution** and circulation time data suggested that the CTLA4Ig-liposomes could be improved upon minimizing the FcR-mediated uptake by Fc receptor-bearing cells. Thus, the strategy of conjugating CTLA4Ig to liposomes could be exploited for immune intervention in transplantation and autoimmune diseases for the efficient blocking of costimulation.

L8 ANSWER 7 OF 14 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2004:25466 Document No.: PREV200400023870. SOMATOSTATIN, VIP AND NEW ANALOGS: ENDOCYTOSIS AND EFFECTS ON SECRETION IN NEUROENDOCRINE CELLS. Grotzinger, Carsten [Reprint Author]; Maecke, Helmut; Prada, Javier; Licha, Kai; Henklein, Peter; Wiedenmann, Bertram. Berlin, Germany. Digestive Disease Week Abstracts and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. M1081. e-file.
Meeting Info.: Digestive Disease 2003. FL, Orlando, USA. May 17-22, 2003. American Association for the Study of Liver Diseases; American Gastroenterological Association; American Society for Gastrointestinal Endoscopy; Society for Surgery of the Alimentary Tract.
Language: English.

AB Background/Objective: The overexpression of peptide receptors has been utilized for several years in the diagnosis of neuroendocrine tumors. Recently, we have introduced near-infrared labelled somatostatin analogues as tracers for such tumors in vitro and in a mouse model. We here report the synthesis optimization of peptide-dye conjugates consisting of near-infrared fluorescent dyes and new somatostatin analogs with a broad spectrum of subtype specificity or VIP receptor **ligands** as contrast agents for optical imaging. Methods: Receptor binding was studied by inhibition assays. Internalization and subcellular localization of the dye conjugates were examined in BON, Rin38 and HT-29 cells, in cells expressing a receptor-EGFP **fusion protein** and in human primary neuroendocrine cells by confocal laser microscopy. Whole-body imaging of nude mouse xenografts was used to assess the conjugates in vivo. Binding and internalization studies were done by laser microscopy and biochemical assays. Results: Fluorescence of the VIP peptide was rapidly eliminated from the cultured cells. **Biodistribution** analysis showed peptide-typic results with rather moderate values in kidney and high uptake in the liver. Whole-body imaging was done in vivo after intravenous injection into tumor-bearing nude mice by laser-induced fluorescence. In these animals, tumor fluorescence increased rapidly. Native peptides were removed from tumors within minutes while stable analogues accumulated over up to 24 hours. Derivatives optimized in an substitution analysis of the VIP molecule yielded somewhat better results but failed to have good imaging properties. Experiments using several well known analogues (octreotide, octreotate) as well as some new broad-band analogs of somatostatin showed that internalization of **ligand** and receptor can differ greatly depending on the receptor subtype as well as the structure of the **ligand.** Structure-function relationships of these **ligands** with regard to endocytosis are discussed in conjunction with physiological

parameters such as influence on secretion of CgA and serotonin from neuroendocrine cells. Conclusion: Improving peptide **ligands** for tumor targeting can be done by rational design or combinatorial approaches. The effect of amino acid substitutions on the properties of the resulting analogues is still not predictable. Universal, "pan"-somatostatin analogs are valuable candidates for several diagnostic as well as therapeutic challenges..

L8 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2007 ACS on STN
2002:716121 Document No. 137:246546 Multispecific **ligands** or antibodies for targeted immunotherapy of autoimmune diseases, cancers, and infections. Herman, William (Can.). PCT Int. Appl. WO 2002072141 A2 20020919, 159 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-CA317 20020311. PRIORITY: US 2001-274217P 20010309; US 2001-276911P 20010320; US 2001-279132P 20010328; US 2001-281029P 20010407; US 2001-306148P 20010719; CA 2002-2368708 20020114.
AB The invention contemplates a composition containing a multispecific **ligand** containing at least a first **ligand** binding moiety and a second **ligand** binding moiety. The first **ligand** binding moiety specifically binds with a pre-selected first affinity to at least a first **ligand.** The first **ligand** has a first **biodistribution.** The second **ligand** binding moiety specifically binds with a pre-selected affinity to at least a second **ligand.** The second **ligand** has a second **biodistribution.** The affinity of first and second **ligand** binding moieties are selected to bias the **biodistribution** of the multispecific **ligand** in favor of a selected location of one or both of the **ligands.** The **ligand** is an antibody or fragment, anti-idiotypic antibody, cell surface marker or antigen or receptor, autoantigen, cytokine receptor, etc. The multispecific **ligands** or antibodies are useful for treating cancer, autoimmune disease, viral infection, or parasitic infection.

L8 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2007 ACS on STN
2002:942802 Document No. 138:19475 Multimeric immunotoxic **fusion proteins** uses in treatment of hyperproliferative disorders such as cancer. Vallera, Daniel A.; Blazar, Bruce R. (Regents of the University of Minnesota, USA). U.S. US 6492498 B1 20021210, 26 pp. (English). CODEN: USXXAM. APPLICATION: US 1999-440344 19991115.
AB The invention features **fusion protein** monomers, multimeric immunotoxic proteins, nucleic acids encoding **fusion protein** monomers, vectors containing the nucleic acids, and cells containing the vectors. Also encompassed by the invention are methods of killing pathogenic cells and making multimeric immunotoxic proteins.

L8 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 4
2002698166. PubMed ID: 12459375. An antibody-calmodulin **fusion protein** reveals a functional dependence between macromolecular isoelectric point and tumor targeting performance. Melkko Samu; Halin Cornelia; Borsi Laura; Zardi Luciano; Neri Dario. (Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology Zurich, Zurich, Switzerland.) International journal of radiation oncology, biology, physics, (2002 Dec 1) Vol. 54, No. 5, pp. 1485-90. Journal code: 7603616. ISSN: 0360-3016. Pub. country: United States. Language: English.
AB PURPOSE: Human monoclonal antibodies are promising agents for the development of improved anticancer therapeutics, because, unlike low-molecular-weight chemotherapeutic agents, they can selectively localize to solid tumors. In particular, the scFv(L19) antibody fragment,

specific for the EDB domain of fibronectin, a marker of angiogenesis, has demonstrated an impressive tumor targeting performance in a variety of tumor-bearing animals and in patients with cancer. The purpose of this study was to develop a tumor pretargeting strategy, based on a novel anti-EDB **fusion protein.** METHODS AND MATERIALS: We have fused the scFv(L19) to calmodulin, a small acidic protein for which specific binding peptides with a dissociation constant in the picomolar range are available. The resulting **fusion protein** has been expressed in mammalian cells and purified to homogeneity, before being characterized by quantitative **biodistribution** analysis in mice bearing the F9 murine teratocarcinoma. RESULTS: Surprisingly, we have found that the fusion of scFv(L19) to calmodulin completely abrogated the tumor targeting ability of the antibody in vivo, although both scFv(L19) and calmodulin moieties within the **fusion protein** retained unaltered binding affinities toward their respective **ligand.** Furthermore, a systematic analysis of 13 derivatives of scFv(L19) recently produced in our laboratories showed that the 10 derivatives that retain the tumor targeting ability of the parental antibody have isoelectric points (pI) between 5.0 and 9.0, whereas scFv(L19)-calmodulin (pI = 4.49) and two other derivatives of scFv(L19) with pI >9.0 were unable to target tumors in vivo. CONCLUSIONS: Because the EDB domain of fibronectin is a component of the modified extracellular matrix, predominantly located at the abluminal side of tumor blood vessels, our data suggest that extreme pI values of antibody-based pharmaceuticals may inhibit protein extravasation, perhaps by virtue of electrostatic interactions with endothelial cells and/or components of the extracellular matrix.

L8 ANSWER 11 OF 14 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

2003:336950 Document No.: PREV200300336950. Comparison of a Tetravalent Single-Chain Antibody-Streptavidin **Fusion Protein** and an Antibody-Streptavidin Chemical Conjugate in Pretargeted Anti-CD20 Radioimmunotherapy of B-Cell Lymphomas. Pagel, John M. [Reprint Author]; Schultz, Jody [Reprint Author]; Axworthy, Donald [Reprint Author]; Hedin, Nathan [Reprint Author]; Theodore, Louis J. [Reprint Author]; Mallet, Robert [Reprint Author]; Wilbur, D. Scott [Reprint Author]; Press, Oliver W. [Reprint Author]. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 2253. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Radioimmunotherapy (RIT) using anti-CD20 monoclonal antibodies (mAb) produces response rates of 60-95% in patients with relapsed non-Hodgkin's lymphoma (NHL); however, tumor-to-normal organ ratios of absorbed radiation are relatively low and many patients relapse. The efficacy of RIT is currently limited by non-specific delivery of radiation to normal tissues as a result of the long circulating half-life of radiolabeled antibodies. We have previously shown that pretargeted RIT using a covalent conjugate of the 1F5 anti-CD20 mAb with streptavidin (SA) significantly augments the efficacy of RIT and decreases the toxicity of therapy in mouse lymphoma xenograft models compared with a conventional, directly labeled 1F5 radioimmunoconjugate. Investigators at NeoRx Corp. independently studied a novel tetravalent single-chain (scFv) anti-CD20-SA **fusion protein** and demonstrated its high antigen-binding avidity, excellent tumor localization and efficient targeting of radiobiotin. In the current study, we have directly compared these two promising new anti-CD20 pretargeting methods. Athymic mice bearing s.c. Ramos lymphoma xenografts received 1.4 nmol of either 1F5-SA or B9E9(scFv)4-SA i.p., followed 24 hours later by 5.8 nmol of a clearing agent (synthetic biotin-N-acetyl-galactosamine) to remove non-localized conjugate from the circulation, followed 2 hours later by 1 mug DOTA-biotin **ligand** labeled with 111In. The

biodistributions of each antibody were evaluated by sacrificing mice (5 per group) at 24, 48, 72, 96, and 144 hours post-injection of therapeutic radiolabeled 111In-DOTA-biotin. Tumors and organs were harvested, weighed, and assayed for 111In activity. Results demonstrated that peak tumor concentrations of the two proteins were similar, but that peak localization occurred slightly earlier with the 1F5-SA conjugate. At 48 hours, 14.1 +- 2.1% of the injected dose of radionuclide was delivered per gram of tumor (% ID/g) using B9E9(scFv)4 compared with 12.2 +- 2.6% ID/g for 1F5-SA after 24 hours. Superior tumor-to-normal organ ratios of radioactivity were consistently seen using the B9E9 tetravalent scFv-SA **fusion protein** compared to the 1F5-SA chemical conjugate. The tumor-to-blood ratios were almost 200:1 after 48 hours with the **fusion protein** compared to <10:1 for the chemical conjugate at the same time point. Therapy experiments testing the efficacy of these proteins in mice bearing lymphoma xenografts demonstrated that complete responses could be routinely obtained using either reagent followed by 800-1200 muCi of 90Y-DOTA-biotin. Doses of 1200 muCi of 90Y-DOTA-biotin cured 100% of mice treated with either B9E9(scFv)4-SA or 1F5-SA whereas all control mice died within 18 days. These data suggest that anti-CD20 pretargeted RIT using either a scFv-SA **fusion protein** (B9E9) or an intact mAb-SA chemical conjugate is highly effective and minimally toxic. Compared to the antibody-SA chemical conjugate however, the genetically engineered **fusion protein** has a well-defined homogeneous composition that is simpler and less expensive to produce. Additional pretargeting RIT studies are underway to further optimize the efficacy and diminish the toxicities of this approach.

L8 ANSWER 12 OF 14 MEDLINE on STN

2000204822. PubMed ID: 10740649. Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL): initial phase I/II study results. Weiden P L; Breitz H B; Press O; Appelbaum J W; Bryan J K; Gaffigan S; Stone D; Axworthy D; Fisher D; Reno J. (Virginia Mason Medical Center, Seattle, WA, USA.) Cancer biotherapy & radiopharmaceuticals, (2000 Feb) Vol. 15, No. 1, pp. 15-29. Journal code: 9605408. ISSN: 1084-9785. Pub. country: United States. Language: English.

AB Pretargeted radioimmunotherapy (PRIT) was investigated in patients with non-Hodgkin's lymphoma (NHL). The PRIT approach used in this study is a multi-step delivery system in which an antibody is used to target streptavidin to a tumor associated antigen receptor, and subsequently biotin is then used to target 90Y radioisotope to the tumor localized streptavidin. A chimeric, IgG1, anti-CD20 antibody, designated C2B8 or Rituximab, was conjugated to streptavidin (SA) and administered to patients with NHL. Thirty-four hours later, a clearing agent, synthetic biotin-N-acetyl-galactosamine, was administered to remove non-localized conjugate from the circulation. Finally, a DOTA-biotin **ligand**, labeled with 111In for imaging and/or 90Y for therapy was administered. Ten patients with relapsed or refractory NHL were studied. In three patients, the C2B8/SA conjugate was radiolabeled with a trace amount of 186Re in order to assess pharmacokinetics and **biodistribution** using gamma camera imaging. Seven patients received 30 or 50 mCi/m2 90Y DOTA-biotin. Re-186 C2B8/SA images confirmed that the conjugate localized to known tumor sites and that the clearing agent removed > 95% of the conjugate from the circulation. Radiolabeled biotin localized well to tumor. Unbound radiobiotin was rapidly excreted from the whole body and normal organs. The mean tumor dose calculated was 29 +/- 23 cGy/mCi 90Y and the average whole body dose was 0.76 +/- 0.3 cGy/mCi 90Y, resulting in a mean tumor to whole body dose ratio of 38:1. Only grade I/II non-hematologic toxicity was observed. Hematologic toxicity was also not severe; i.e., five of the seven patients who received 30 or 50 mCi/m2 of 90Y-DOTA-biotin experienced only transient grade III (but no grade IV) hematologic toxicity. Although six of ten patients developed humoral immune responses to the streptavidin, these were delayed and transient and hence may not preclude retreatment. Six of seven patients who received 30 or 50mCi/m2 90Y achieved objective tumor regression, including three

complete and one partial response. The estimate of tumor to whole body dose ratio (38:1) achieved with PRIT in these NHL patients is higher than has been achieved in other studies using conventional RIT. Toxicity was mild and tumor response encouraging. PRIT clearly deserves additional study in patients with NHL.

L8 ANSWER 13 OF 14 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1999:584355 The Genuine Article (R) Number: 220MJ. Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis. McCall A M; Adams G P; Amoroso A R; Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; Marks J D; Weiner L M (Reprint). Fox Chase Canc Ctr, 7701 Burholme Ave, Philadelphia, PA 19111 USA (Reprint); Fox Chase Canc Ctr, Philadelphia, PA 19111 USA; San Francisco Gen Hosp, Dept Anesthesia, San Francisco, CA 94110 USA. MOLECULAR IMMUNOLOGY (MAY 1999) Vol. 36, No. 7, pp. 433-445. ISSN: 0161-5890. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Bispecific antibody (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal antibody (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of antibody Fc domains with cellular Fc receptors has limited the doses of antibody (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the HER2/neu protooncogene product and the human Fc gamma RIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from single-chain Fv (scFv) molecules specific for HER2/neu and CD16. The human anti-CD16 scFv component, NM3E2, was: isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fe binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/neu scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as fusion partner for the creation of a bispecific scFv (bs-scFv). In the presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian cancer cells overexpressing HER2/neu. **Biodistribution** studies performed in SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of I-125-labeled C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the **fusion protein.** This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5 x NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins with potential clinical applications as a result of its human origin, lack of an Fe domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting. (C) 1999 Elsevier Science Ltd. All rights reserved.

L8 ANSWER 14 OF 14 MEDLINE on STN

94289272. PubMed ID: 8018538. Comparative **biodistributions** of indium-111-labelled macrocycle chimeric B72.3 antibody conjugates in tumour-bearing mice. Turner A; King D J; Farnsworth A P; Rhind S K; Pedley R B; Boden J; Boden R; Millican T A; Millar K; Boyce B; +. (Celltech Research, Slough, Berkshire, UK.) British journal of cancer, (1994 Jul) Vol. 70, No. 1, pp. 35-41. Journal code: 0370635. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB A novel 111In **ligand** (a C-functionalised derivative of 1,4,7-triazacyclononanetriacetic acid), termed 9N3, was covalently attached to chimeric B72.3, labelled with 111In and compared with

111In-labelled chimeric B72.3 diethylenetriaminepentaacetic acid (DTPA) cyclic anhydride conjugate (cDTPA) and a C-linked derivative of DTPA (CT-DTPA) in athymic mice bearing human colon carcinoma xenografts. Significant differences in **biodistribution** were observed between 9N3 and cDTPA conjugates especially in the tumour uptake and blood, liver, femur and colon levels at 24, 48 and 144 h. Significantly higher tumour uptake was observed for 111In-cB72.3-9N3 compared with 111In-cB72.3-cDTPA at all time points. Radiolocalisation (RI) indices increased with time for the 9N3 conjugate but remained constant for the cDTPA conjugate. The **biodistribution** of 111In-labelled cB72.3-CT-DTPA was similar to that of 111In-labelled cB72.3-9N3 except for elevated kidney levels. A 12N4 macrocycle (a C-functionalised derivative of 1,4,7,10-tetraazacyclododecanetetraacetic acid) was also tested for its ability to chelate 111In and its **biodistribution** examined. Labelled conjugates with this macrocycle were more difficult to prepare in a stable form but gave a very similar **biodistribution** to the 9N3 macrocycle conjugate. Macrocycle-antibody conjugates of this type offer considerable promise for tumour imaging in patients.

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L10          91 L5 AND CXCR4

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L11          17 L10 AND AFFINITY

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L12           5 L11 AND PD<20020114

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L12  ANSWER 1 OF 5     MEDLINE on STN
2002436211.    PubMed ID: 12193720.   RGS13 regulates germinal center B
     lymphocytes responsiveness to CXC chemokine ligand (CXCL)12 and
     CXCL13. Shi Geng-Xian; Harrison Kathleen; Wilson Gaye Lynn; Moratz
     Chantal; Kehrl John H. (B Cell Molecular Immunology Section, Laboratory of
     Immunoregulation, National Institute of Allergy and Infectious Diseases,
     National Institutes of Health, Bethesda, MD 20892, USA. ) Journal of
     immunology (Baltimore, Md. : 1950), (2002 Sep 1) Vol. 169, No.
     5, pp. 2507-15.  Journal code: 2985117R. ISSN: 0022-1767. Pub. country:
     United States. Language: English.
AB   Normal lymphoid tissue development and function depend upon directed cell
     migration.  Providing guideposts for cell movement and positioning within
     lymphoid tissues, chemokines signal through cell surface receptors that
     couple to heterotrimeric G proteins, which are in turn subject to
     regulation by regulator of G protein signaling (RGS) proteins.  In this
     study, we report that germinal center B lymphocytes and thymic epithelial
     cells strongly express one of the RGS family members, RGS13.  Located
     between Rgs1 and Rgs2, Rgs13 spans 42 kb on mouse chromosome 1.  Rgs13
     encodes a 157-aa protein that shares 82% amino acid identity with its
     159-aa human counterpart.  In situ hybridization with sense and antisense
     probes localized Rgs13 expression to the germinal center regions of mouse
     spleens and Peyer's patches and to the thymus medulla.  Affinity
     -purified RGS13 Abs detected RGS13-expressing cells in the light zone of
     the germinal center.  RGS13 interacted with both Gialpha and Gqalpha and
     strongly impaired signaling through G(i)-linked signaling pathways,
     including signaling through the chemokine receptors CXCR4 and
     CXCR5.  Prolonged CD40 signaling up-regulated RGS13 expression in human
     tonsil B lymphocytes.  These results plus previous studies of RGS1
     indicate the germinal center B cells use two RGS proteins, RGS1 and RGS13,
     to regulate their responsiveness to chemokines.

L12  ANSWER 2 OF 5     MEDLINE on STN
1998298084.    PubMed ID: 9632631.   Spontaneous and ligand-induced
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trafficking of CXC-chemokine receptor 4. Tarasova N I; Stauber R H; Michejda C J. (Molecular Aspects of Drug Design Section Applied Biosystems Laboratories Basic Research Program, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702, USA.. Tarasova@ncifcrf.gov) . The Journal of biological chemistry, (**1998 Jun 26**) Vol. 273, No. 26, pp. 15883-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A chimeric protein consisting of CXC-chemokine receptor 4 (**CXCR4**) and the green fluorescent protein (GFP) was used for studying receptor localization and trafficking in real time in stably transduced HeLa, U-937, CEM, and NIH/3T3 cells. **CXCR4**-GFP was fully active as a co-receptor in mediating human immunodeficiency virus (HIV) entry. Both **CXCR4** and **CXCR4**-GFP were found to undergo significant spontaneous endocytosis. Only 51.5 +/- 7.8% of receptor molecules were found on the plasma membrane in CD4-positive cells, 43.9 +/- 8.5% were found in CD4-negative HeLa cells, 75.6 +/- 9.7% were found in U-937 cells, 72.5 +/- 7.9 were found in CEM cells, and almost none were found in in NIH/3T3 cells. Stromal cell-derived factor-1alpha induced rapid endocytosis of cell surface receptor molecules. A significant part of **CXCR4** was targeted to lysosomes upon binding of the **ligands**, and recycling of internalized **CXCR4** was not efficient. Only about 30% of receptor molecules recycled back to the cell surface in HeLa cells, 5% recycled in U937, and 10% recycled in CEM cells, suggesting that the protective effect of chemokines against HIV infection can be attributed not only to competition for binding but also to depletion of the co-receptor molecules from the cell surface. Envelope glycoprotein gp120 of syncytia-inducing/lymphocyte tropic HIV-1 strains induced rapid internalization of **CXCR4** in both CD4-negative and CD4-positive cells, suggesting that gp120 is a high **affinity ligand** of **CXCR4.**

L12 ANSWER 3 OF 5 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:342431 Document No.: PREV199800342431. Spontaneous and **ligand** -induced trafficking of CXC-chemokine receptor 4. Tarasova, Nadya I. [Reprint author]; Stauber, Roland H.; Michejda, Christopher J.. Molecular Aspects Drug Design Section ABL-Basic Res. Program, Natl. Cancer Inst., Frederick Cancer Res. Development Cent., P.O. Box B, Frederick, MD 21702, USA. Journal of Biological Chemistry, (**June 26, 1998**) Vol. 273, No. 26, pp. 15883-15886. print.
CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

AB A chimeric protein consisting of CXC-chemokine receptor 4 (**CXCR4**) and the green fluorescent protein (GFP) was used for studying receptor localization and trafficking in real time in stably transduced HeLa, U-937, CEM, and NIH/3T3 cells. **CXCR4**-GFP was fully active as a co-receptor in mediating human immunodeficiency virus (HIV) entry. Both **CXCR4** and **CXCR4**-GFP were found to undergo significant spontaneous endocytosis. Only 51.5 +- 7.8% of receptor molecules were found on the plasma membrane in CD4-positive cells, 43.9 +- 8.5% were found in CD4-negative HeLa cells, 75.6 +- 9.7% were found in U-937 cells, 72.5 +- 7.9 were found in CEM cells, and almost none were found in in NIH/3T3 cells. Stromal cell-derived factor-1alpha induced rapid endocytosis of cell surface receptor molecules. A significant part of **CXCR4** was targeted to lysosomes upon binding of the **ligands**, and recycling of internalized **CXCR4** was not efficient. Only about 30% of receptor molecules recycled back to the cell surface in HeLa cells, 5% recycled in U937, and 10% recycled in CEM cells, suggesting that the protective effect of chemokines against HIV infection can be attributed not only to competition for binding but also to depletion of the co-receptor molecules from the cell surface. Envelope glycoprotein gp120 of syncytia-inducing/lymphocyte tropic HIV-1 strains induced rapid internalization of **CXCR4** in both CD4-negative and CD4-positive cells, suggesting that gp120 is a high **affinity ligand** of **CXCR4.**

L12 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN

2002:490348 Document No. 137:277518 Expression and purification of recombinant human chemokine SDF-1β in E. coli. Zheng, Hong; Peiper, Stephen C.; Zhu, Xi-hua (Department of Immunology, Third Military Medical University, Chungking, 400038, Peop. Rep. China). Journal of Medical Colleges of PLA, 17(1), 24-28 (English) **2002.** CODEN: JMCPE6. ISSN: 1000-1948. Publisher: Journal of Medical Colleges of PLA, Editorial Board.

AB Objective: To obtain recombinant human SDF-1β expressed in E. coli and purify SDF-1β with biol. activity from the bacterium. Methods: A thioredoxin-SDF-1β **fusion protein** (26+103) composed of 230 amino acid residues was expressed in E. coli AD494 (DE3) pLysS under the induction of IPTG when pET32a(+)-SDF-1β was used as an expression vector. Purified SDF-1β was produced through following procedures: Bacteria lysis, metal-chelated **affinity** chromatog. (MAC), enterokinase digestion to sep. SDF-1β from **fusion protein**, cation exchange chromatog. (CEC) and reverse-phase high performance liquid chromatog. (RP-HPLC). Western blot with anti-SDF-1β monoclonal antibody (mAb), N-terminal amino acid sequencing, **ligand**-binding assay and cytosensor/microphysiometry were used to investigate the biochem. characters and biol. activities of the purified SDF-1β. Results: From 10% to 15% of total bacterium protein was expressed as **fusion protein.** Approx. 400 μg purified SDF-1β (7.8+103) consisting of 71 amino acid residues were produced from 1 L of fermented bacteria. Western blot showed that anti-SDF-1β mAb bound with the purified SDF-1β specifically. N-terminal amino acid sequencing indicates that N-terminus of purified SDF-1β possessed the same amino acid sequence as natural one. Purified SDF-1β not only had the binding activity with **CXCR4** expressing cells [Kd=(12.20) nmol/L], but also activated **CXCR4** expressing cell signaling specifically in a dose-dependence manner. Conclusion: The purified recombinant human SDF-1β produced with this method possesses biochem. characters and biol. activities as same as those nature human SDF-1β.

L12 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN

2001:328729 Document No. 135:353460 Expression and purification of recombinant human chemokine SDF-1β in Escherichia coli. Zheng, Hong; Peiper, Stephen C.; Zhu, Xihua (Department of Immunology, Third Military Medical University, Chungking, 400038, Peop. Rep. China). Di-San Junyi Daxue Xuebao, 23(1), 62-65 (Chinese) **2001.** CODEN: DYXUE8. ISSN: 1000-5404. Publisher: Di-San Junyi Daxue.

AB A thioredoxin-SDF-1β **fusion protein** (2.6 x 104) composed of 230 amino acid residues was prepared by expression of pET32a(+)-SDF-1β in E. coli AD494(DE3)pLyS under induction of IPTG. The recombinant SDF-1β was separated and purified by bacterial lysis, metal-chelated **affinity** chromatog. (MAC), enterokinase digestion, cation exchange chromatog. (CEC), and RP-HPLC. The biochem. characteristics and biol. activities of the purified SDF-1β were determined by Western blotting with anti-SDF-1β monoclonal antibody (mAb), N-terminal amino acid sequencing, **ligand**-binding assay, and Cytosensor/microphysiometry. About 10%-15% of total bacterium protein was expressed as **fusion protein.** Approx. 400 μg purified SDF-1β (7.8 x 103) composed of 71 amino acid residues were produced from 1 L fermented bacteria. Anti-SDF-1β mAb could bind specifically with the purified SDF-1β. N-Terminus of purified SDF-1β had the same amino acid sequence as the natural one. Purified SDF-1β could bind with **CXCR4** expression cells [Kd = (12.20 ± 2.99) nM] and dose-dependently activate **CXCR4** expressing cell signaling. The results showed that the recombinant human SDF-1β had the same biochem. characteristics and biol. activities as the natural one.

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L13 183 L5 AND BISPECIFIC

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L16           1 L15 AND PD<20020114

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L16  ANSWER 1 OF 1  SCISEARCH  COPYRIGHT (c) 2007 The Thomson Corporation  on
     STN
1999:584355   The Genuine Article (R) Number: 220MJ. Isolation and
     characterization of an anti-CD16 single-chain Fv fragment and construction
     of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers
     CD16-dependent tumor cytolysis.  McCall A M; Adams G P; Amoroso A R;
     Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; Marks J D; Weiner L M
     (Reprint). Fox Chase Canc Ctr, 7701 Burholme Ave, Philadelphia, PA 19111
     USA (Reprint); Fox Chase Canc Ctr, Philadelphia, PA 19111 USA; San
     Francisco Gen Hosp, Dept Anesthesia, San Francisco, CA 94110 USA.
     MOLECULAR IMMUNOLOGY (MAY 1999) Vol. 36, No. 7, pp. 433-445.
     ISSN: 0161-5890. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD,
     LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        Bispecific antibody (bsAb)-based clinical trials of cancer
     have been conducted primarily using intact murine monoclonal antibody
     (mAb)-derived molecules.  In some of these trials, toxicity resulting from
     the interactions of antibody Fc domains with cellular Fc receptors has
     limited the doses of antibody (Ab) that can be employed.  Furthermore,
     human anti-mouse Ab responses prohibit multiple therapy courses.  These
     factors have decreased the efficacy of the bsAb 2B1, which targets the
     extracellular domains (ECD) of the HER2/neu protooncogene product and the
     human Fc gamma RIII (CD16).  To address these obstacles, we have
     constructed and characterized a fully human gene-fused bsAb from
     single-chain Fv (scFv) molecules specific for HER2/neu and CD16.  The
     human anti-CD16 scFv component, NM3E2, was: isolated from a human scFv
     phage display library.  As binding of NM3E2 to human neutrophil-associated
     CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2
     recognizes an epitope in the vicinity of the Fe binding pocket.
     Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based
     epitope mapping to share an overlapping epitope with the Leu-11c mAb.  The
     human anti-HER2/neu scFv component, C6.5, which was previously isolated
     from a human scFv phage display library, was employed as fusion partner
     for the creation of a bispecific scFv (bs-scFv).  In the
     presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes
     promoted significant lysis of human SK-OV-3 ovarian cancer cells
     overexpressing HER2/neu.  Biodistribution studies performed in
     SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of I-125-labeled
     C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following
     injection.  These results indicated that both scFv components of the
     bs-scFv retained their function in the fusion protein.
     This bsAb should overcome some of the problems associated with the 2B1
     bsAb.  C6.5 x NM3E2 bs-scFv offers promise as a platform for
     multifunctional binding proteins with potential clinical applications as a
     result of its human origin, lack of an Fe domain, ease of production, high
     level of in vitro tumor cell cytotoxicity and highly selective tumor
     targeting. (C) 1999 Elsevier Science Ltd.  All rights reserved.


=> s l13 and different affinity
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L17 0 L13 AND DIFFERENT AFFINITY

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L18 62 L13 AND AFFINITY

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L20 15 L19 AND PD<20020114

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L20 ANSWER 1 OF 15 MEDLINE on STN
2002678690. PubMed ID: 12438615. Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked **ligands.** Ponnazhagan Selvarangan; Mahendra Gandham; Kumar Sanjay; Thompson John A; Castillas Mark Jr. (Department of Pathology, LHRB 513, The University of Alabama at Birmingham, 701 19th Street South, Birmingham, AL 35294, USA.. sponnazh@path.uab.edu) . Journal of virology, **(2002 Dec)** Vol. 76, No. 24, pp. 12900-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The development of targeted vectors, capable of tissue-specific transduction, remains one of the important aspects of vector modification for gene therapy applications. Recombinant adeno-associated virus type 2 (rAAV-2)-based vectors are nonpathogenic, have relatively low immunogenicity, and are capable of long-term transgene expression. AAV-2 vectors bind primarily to heparan sulfate proteoglycan (HSPG), a receptor that is present in many tissues and cell types. Because of the widespread expression of HSPG on many tissues, targeted transduction in vivo appears to be limited with AAV-2 vectors. Thus, development of strategies to achieve transductional targeting will have a profound benefit in the future application of these vectors. We report here a novel conjugate-based targeting method to enhance tissue-specific transduction of AAV-2-based vectors. The present report utilized a high-**affinity** biotin-avidin interaction as a molecular bridge to cross-link purified targeting **ligands**, produced genetically as **fusion proteins** to core-streptavidin, in a prokaryotic expression system. Conjugation of the **bispecific** targeting protein to the vector was achieved by biotinylating purified rAAV-2 without abolishing the capsid structure, internalization, and subsequent transgene expression. The tropism-modified vectors, targeted via epidermal growth factor receptor (EGFR) or fibroblast growth factor 1alpha receptor (FGFR1alpha), resulted in a significant increase in transduction efficiency of EGFR-positive SKOV3.ip1 cells and FGFR1alpha-positive M07e cells, respectively. Further optimization of this method of targeting should enhance the potential of AAV-2 vectors in ex vivo and in vivo gene therapy and may form the basis for developing targeting methods for other AAV serotype capsids.

L20 ANSWER 2 OF 15 MEDLINE on STN
2002411062. PubMed ID: 12165442. Fab-scFv **fusion protein** : an efficient approach to production of **bispecific** antibody fragments. Lu Dan; Jimenez Xenia; Zhang Haifan; Bohlen Peter; Witte Larry; Zhu Zhenping. (Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) Journal of immunological methods, **(2002 Sep 15)** Vol. 267, No. 2, pp. 213-26. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.
AB The clinical development of **bispecific** antibodies (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel

bispecific antibody fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step **affinity** chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two antibodies directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific** antibody fragments not only retained the antigen-binding capacity of each of the parent antibodies, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific** antibodies were comparable to their parent antibodies in their potency in blocking **ligand** binding to the receptors and in inhibiting **ligand**-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L20 ANSWER 3 OF 15 MEDLINE on STN

2002083005. PubMed ID: 11809717. Adenovirus targeting to c-erbB-2 oncoprotein by single-chain antibody fused to trimeric form of adenovirus receptor ectodomain. Kashentseva Elena A; Seki Toshiro; Curiel David T; Dmitriev Igor P. (Division of Human Gene Therapy, Department of Medicine, University of Alabama at Birmingham, 35294-3300, USA.) Cancer research, **(2002 Jan 15)** Vol. 62, No. 2, pp. 609-16. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB The use of adenovirus (Ad) vectors for cancer gene therapy applications is currently limited by several factors, including broad Ad tropism associated with the widespread expression of coxsackievirus and adenovirus receptor (CAR) in normal human tissues, as well as limited levels of CAR in tumor cells. To target Ad to relevant cell types, we have proposed using soluble CAR (sCAR) ectodomain fused with a **ligand** to block CAR-dependent native tropism and to simultaneously achieve infection through a novel receptor overexpressed in target cells. To confer Ad targeting capability on cancer cells expressing the c-erbB-2/HER-2/neu oncogene, we engineered a **bispecific** adapter protein, sCARfC6.5, that consisted of sCAR, phage T4 fibritin polypeptide, and C6.5 single-chain fragment variable (scFv) against c-erbB-2 oncoprotein. Incorporation of fibritin polypeptide provided trimerization of sCAR **fusion proteins** that, compared with monomeric sCAR protein, resulted in augmented **affinity** to Ad fiber knob domain and in increased ability to block CAR-dependent Ad infection. We demonstrated that sCARfC6.5 protein binds to cellular c-erbB-2 oncoprotein and mediates efficient Ad targeting via a CAR-independent pathway. As illustrated in cancer cell lines that overexpress c-erbB-2, targeted Ad, complexed with sCARfC6.5 adapter protein, provided from 1.5- to 17-fold enhancement of gene transfer compared with Ad alone and up to 130-fold increase in comparison with untargeted Ad complexed with sCARf control protein. The use of recombinant trimeric sCAR-scFv adapter proteins may augment Ad vector potency for targeting cancer cell types.

L20 ANSWER 4 OF 15 MEDLINE on STN

1998298518. PubMed ID: 9634779. Calmodulin as a versatile tag for antibody fragments. Neri D; de Lalla C; Petrul H; Neri P; Winter G. (Cambridge Centre for Protein Engineering, MRC Centre, UK.) Bio/technology (Nature Publishing Company), **(1995 Apr)** Vol. 13, No. 4, pp. 373-7. Journal code: 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.

AB Calmodulin is a highly acidic protein (net charge -24 at pH 8.0 in the absence of calcium) that binds to peptide and organic **ligands** with high **affinity** (Ka > 10(9) M-1) in a calcium-dependent manner. We have exploited these properties to develop calmodulin as a versatile tag for antibody fragments. Fusions of calmodulin with single chain Fv fragments (scFv) could be expressed by secretion from bacteria in good yield (5-15 mg/l in shaker flasks), and purified from periplasmic lysates or broth to homogeneity in a single step, either by binding to

anion-exchange resin (DEAE-Sephadex), or to an organic **ligand** of calmodulin (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide-agarose). The antibody fusions could be detected by binding of fluorescently labeled peptide **ligands**, as illustrated by their use in confocal microscopy, fluorescent activated cell sorting and "band shift" gel electrophoresis. Moreover, the interaction between calmodulin and peptide **ligands** could provide a means of heterodimerization of proteins, as illustrated by the assembly of an antibody-calmodulin fusion with maltose binding protein tagged with a peptide **ligand** of calmodulin.

L20 ANSWER 5 OF 15 MEDLINE on STN

97450548. PubMed ID: 9305536. Linear gene fusions of antibody fragments with streptavidin can be linked to biotin labelled secondary molecules to form **bispecific** reagents. Pearce L A; Oddie G W; Coia G; Kortt A A; Hudson P J; Lilley G G. (CSIRO, Division of Biomolecular Engineering, Parkville, Australia.) Biochemistry and molecular biology international, **(1997 Sep)** Vol. 42, No. 6, pp. 1179-88. Journal code: 9306673. ISSN: 1039-9712. Pub. country: Australia. Language: English.

AB Monomeric single chain antibody (scFv) fragments lack both the avidity of the bivalent IgG, or (Fab')2 fragment, and the effector functions conferred by the Fc domain. For certain diagnostic or therapeutic applications it may be desirable to link these molecules to other proteins, antibodies, enzymes or peptide **ligands**, and chemical or recombinant methods have been developed to produce many of these crosslinked reagents. One approach has been to link an antibody fragment to streptavidin which can bind a second biotinylated molecule to create a higher **affinity**, bifunctional or **bispecific** molecule. To demonstrate the applicability of this technology, an anti-neuraminidase NC10 scFv-streptavidin fusion was expressed in E. coli and the product was refolded and purified to homogeneity from 6 M guanidine hydrochloride. Analysis in a BIAcore biosensor showed that the NC10 scFv moiety reacted with immobilised neuraminidase and that the core streptavidin moiety was able to bind biotinylated anti-ferritin Fab' to produce a new model **bispecific** reagent which bound ferritin. Conceptually, this design principle can be applied to the creation of useful diagnostic and possibly therapeutic molecules.

L20 ANSWER 6 OF 15 MEDLINE on STN

97203117. PubMed ID: 9050835. "Peptabody": a new type of high avidity binding protein. Terskikh A V; Le Doussal J M; Crameri R; Fisch I; Mach J P; Kajava A V. (Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland.) Proceedings of the National Academy of Sciences of the United States of America, **(1997 Mar 4)** Vol. 94, No. 5, pp. 1663-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A new type of high avidity binding molecule, termed "peptabody" was created by harnessing the effect of multivalent interaction. A short peptide **ligand** was fused via a semi-rigid hinge region with the coiled-coil assembly domain of the cartilage oligomeric matrix protein, resulting in a pentameric multivalent binding molecule. In the first peptabody (Pab-S) described here, a peptide (S) specific for the mouse B-cell lymphoma BCL1 surface Ig idiotype, was selected from a phage display library. A fusion gene was constructed encoding peptide S, followed by the 24 aa hinge region from camel IgG and a modified 55 aa cartilage oligomeric matrix protein pentamerization domain. The Pab-S **fusion protein** was expressed in Escherichia coli in a soluble form at high levels and purified in a single step by metal-**affinity** chromatography. Pab-S specifically bound the BCL1 surface idiotype with an avidity of about 1 nM, which corresponds to a 2 x 10(5)-fold increase compared with the **affinity** of the synthetic peptide S itself. Biochemical characterization showed that Pab-S is a stable homopentamer of about 85 kDa, with interchain disulfide bonds. Pab-S can be dissociated under denaturing and reducing conditions and reassociated as a pentamer with full-binding activity. This intrinsic

feature provides an easy way to combine Pab molecules with two different peptide specificities, thus producing heteropentamers with **bispecific** and/or chelating properties.

L20 ANSWER 7 OF 15 MEDLINE on STN
97187689. PubMed ID: 9035142. Design and production of novel tetravalent **bispecific** antibodies. Coloma M J; Morrison S L. (Department of Microbiology and Molecular Genetics, University of California at Los Angeles 90095, USA.) Nature biotechnology, **(1997 Feb)** Vol. 15, No. 2, pp. 159-63. Journal code: 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.
AB We have produced novel **bispecific** antibodies by fusing the DNA encoding a single chain antibody (ScFv) after the C terminus (CH3-ScFv) or after the hinge (Hinge-ScFv) with an antibody of a different specificity. The **fusion protein** is expressed by gene transfection in the context of a murine variable region. Transfectomas secrete a homogeneous population of the recombinant antibody with two different specificities, one at the N terminus (anti-dextran) and one at the C terminus (anti-dansyl). The CH3-ScFv antibody, which maintains the constant region of human IgG3, has some of the associated effector functions such as long half-life and Fc receptor binding. The Hinge-ScFv antibody which lacks the CH2 and CH3 domains has no known effector functions.

L20 ANSWER 8 OF 15 MEDLINE on STN
97146059. PubMed ID: 8993006. Cytolytic and cytostatic properties of an anti-human Fc gammaRI (CD64) x epidermal growth factor **bispecific fusion protein.** Goldstein J; Graziano R F; Sundarapandiyan K; Somasundaram C; Deo Y M. (Medarex, Inc., Annandale, NJ 08801, USA.) Journal of immunology (Baltimore, Md. : 1950), **(1997 Jan 15)** Vol. 158, No. 2, pp. 872-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB A **bispecific fusion protein** (H22-EGF) that binds simultaneously to the epidermal growth factor receptor (EGF-R) and to the high **affinity** receptor for the Fc portion of human IgG, Fc gammaRI (CD64), has been successfully constructed and expressed. For this construction, genomic DNA encoding the Fd fragment of humanized anti-Fc gammaRI mAb, H22, which binds Fc gammaRI at an epitope that is distinct from the Fc binding site, was fused to cDNA encoding human epidermal growth factor (EGF), a natural **ligand** for EGF-R. The resulting H22Fd-EGF-expressing vector was transfected into a myeloma cell line that was transfected previously with a vector containing DNA encoding the H22 kappa-light chain. SDS-PAGE analysis of purified H22-EGF demonstrated that the **fusion protein** was secreted predominantly as H22Fab'-EGF monomer (approximately 55 kDa), even though a free Cys residue exists in the hinge region of the H22 Fab' component. Using a novel **bispecific** flow cytometry-binding assay, we demonstrated that the purified **bispecific fusion protein**, H22-EGF, was able to bind simultaneously to soluble Fc gammaRI and EGF-R-expressing cells. H22-EGF inhibited the growth of EGF-R-overexpressing tumor cells and mediated dose-dependent cytotoxicity of these cells in the presence of Fc gammaRI-bearing cytotoxic effector cells. These results suggest that this **fusion protein** may have therapeutic utility for EGF-R-overexpressing malignancies.

L20 ANSWER 9 OF 15 MEDLINE on STN
97110906. PubMed ID: 9005442. **Affinity** enhancement of a recombinant antibody: formation of complexes with multiple valency by a single-chain Fv fragment-core streptavidin fusion. Kipriyanov S M; Little M; Kropshofer H; Breitling F; Gotter S; Dubel S. (Recombinant Antibody Research Group, Heidelberg, Germany.) Protein engineering, **(1996 Feb)** Vol. 9, No. 2, pp. 203-11. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.
AB In antigen-antibody interactions, the high avidity of antibodies depends on the **affinity** and number of the individual binding sites. To

develop artificial antibodies with multiple valency, we have fused the single-chain antibody Fv fragments to core streptavidin. The resulting **fusion protein**, termed scFv::strep, was found after expression in Escherichia coli in periplasmic inclusion bodies. After purification of the recombinant product by immobilized metal **affinity** chromatography, refolding and size-exclusion FPLC, tetrameric complexes resembling those of mature streptavidin were formed. The purified tetrameric scFv::strep complexes demonstrated both antigen- and biotin-binding activity, were stable over a wide range of pH and did not dissociate at high temperatures (up to 70 degrees C). Surface plasmon resonance measurements in a BIAlite system showed that the pure scFv::strep tetramers bound immobilized antigen very tightly and no dissociation was measurable. The association rate constant for scFv::strep tetramers was higher than those for scFv monomers and dimers. This was also reflected in the apparent constants, which was found to be 35 times higher for pure scFv::strep tetramers than monomeric single-chain antibodies. We could also show that most of biotin binding sites were accessible and not blocked by biotinylated E.coli proteins or free biotin from the medium. These sites should therefore facilitate the construction of **bispecific** multivalent antibodies by the addition of biotinylated **ligands**.

L20 ANSWER 10 OF 15 MEDLINE on STN

94239531. PubMed ID: 8183373. Heterodimerization of the IL-2 receptor beta- and gamma-chain cytoplasmic domains is required for signalling. Nakamura Y; Russell S M; Mess S A; Friedmann M; Erdos M; Francois C; Jacques Y; Adelstein S; Leonard W J. (Section on Pulmonary and Molecular Immunology, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892.) Nature, **(1994 May 26)** Vol. 369, No. 6478, pp. 330-3. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The interaction of interleukin-2 (IL-2) and IL-2 receptors critically regulates the T-cell immune response following antigen activation. IL-2 can signal through high or intermediate **affinity** receptors which contain IL-2R alpha (refs 3, 4) +beta (refs 5-8) +gamma (reference 9) or beta+gamma chains, respectively. IL-2R gamma is a common gamma chain, gamma c, also shared by the IL-7 (reference 10) and IL-4 (refs 11, 12) receptors, which when mutated results in X-linked severe combined immunodeficiency. Using chimaeric receptor constructs together with monoclonal or **bispecific** antibodies we demonstrate here that IL-2 signalling requires **ligand**-induced extracellular-domain-mediated heterodimerization of the beta- and gamma c-chain cytoplasmic domains. Anti-IL-2R alpha monoclonal antibodies trigger proliferation of cells transfected with chimaeric constructs in which the extracellular domains of IL-2R beta and gamma c are replaced by that of IL-2R alpha. Other experiments using chimaeric constructs indicated that IL-2 binds monomerically and monovalently to IL-2R alpha and that the beta-transmembrane domain is not required for receptor chain interactions. Finally, we provide a method for mapping residues in the gamma c cytoplasmic domain even in cells that constitutively express gamma c.

L20 ANSWER 11 OF 15 MEDLINE on STN

93246675. PubMed ID: 8482850. Construction of a **bispecific** antibody reacting with the alpha- and beta-chains of the human IL-2 receptor. High **affinity** cross-linking and high anti-proliferative efficiency. Francois C; Boeffard F; Kaluza B; Weidle U H; Jacques Y. (Institut National de la Sante et de la Recherche Medicale (INSERM U211), Nantes, France.) Journal of immunology (Baltimore, Md. : 1950), **(1993 May 15)** Vol. 150, No. 10, pp. 4610-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB A **bispecific** antibody recognizing both the alpha- and beta-chains of the IL-2R was generated by sulfhydryl-directed chemical reassociation of monovalent Fab' fragments prepared from the anti-alpha mAb 33B3.1 (rat IgG2a) and from the anti-beta mAb A41 (mouse IgG1).

Whereas the 33B3.1/A41 **bispecific** mAb (bi-mAb) binds to isolated alpha- and beta-chains with low **affinity** (Kd = 4 nM), its binding to cells co-expressing the two chains shows both low and high **affinity** components. The high **affinity**-binding sites (Kd = 100 pM) most probably correspond to the cross-linking by the bi-mAb of alpha- and beta-chains, whereas the low **affinity** component corresponds to the excess of alpha-chains. High **affinity** binding of bi-mAb on activated T cells is observed at 37 degrees C and not at 4 degrees C, suggesting that i) the two chains are dissociated at 4 degrees C in the absence of **ligand** and ii) the mechanism of bi-mAb catalyzed cross-linking of these two chains is temperature dependent. In contrast to parental 33B3.1 and A41 IgG, which recognize single positive (alpha + and beta +, respectively) and double positive alpha +/beta + cells with similar **affinities**, the 33B3.1/A41 bi-mAb is specific for activated alpha +/beta + cells with respect to its high **affinity** binding. In contrast to A41, which does not affect IL-2-induced proliferation of 4AS cells or anti-CD3-activated PBL, and to 33B3.1, which do inhibit proliferation but only partially and at high doses, the bi-mAb showed full blocking efficiencies at low concentrations (IC50 of 300 to 400pM) corresponding to the formation of high **affinity** alpha/bi-mAb/beta complexes. These half-maximal effects were observed at 10-fold lower concentrations than when using a combination of equimolar concentrations of parental 33B3.1 and A41 IgG. Because of their specificity and high blocking efficiencies, anti-alpha/anti-beta bi-mAb may constitute a better alternative for IL-2R-directed immunosuppression.

L20 ANSWER 12 OF 15 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

2002:9667 Document No.: PREV200200009667. Duocalins: Engineered **ligand** -binding proteins with dual specificity derived from the lipocalin fold. Schlehuber, Steffen; Skerra, Arne [Reprint author]. Lehrstuhl fuer Biologische Chemie, Technische Universitaet Muenchen, D-85350, Freising-Weihenstephan, Germany. Biological Chemistry, (**September, 2001**) Vol. 382, No. 9, pp. 1335-1342. print.
ISSN: 1431-6730. Language: English.

AB Anticalins comprise a novel class of receptor proteins with predetermined **ligand** specificities which were engineered using the lipocalin fold. Attractive features of these artificial **ligand**-binding proteins include their small size and monomeric nature, being composed of a single polypeptide chain. Here we report the construction of a functional **fusion protein** from two independent anticalins, a so-called duocalin. The gene for the **fusion protein** was assembled from nucleotide sequences encoding an anticalin with fluorescein specificity on the one hand and an anticalin with digoxigenin specificity on the other. Both engineered lipocalins were previously selected from a random library prepared on the basis of the bilin-binding protein, a natural lipocalin abundant in insects. The corresponding **fusion protein** was expressed in a secretable form in E. coli cells and isolated from the periplasmic fraction using the Strep-tag method. The major fraction of the purified protein appeared to possess the proper pattern of altogether four disulphide bonds. The **ligand**-binding behaviour of the **fusion protein** was investigated both by solid phase ELISA and in fluorescence titration experiments. Our results demonstrate that the novel **fusion protein** has retained both **ligand** specificities. Up to now, dimerized **ligand** -binding proteins were mostly derived from recombinant antibody fragments. Compared with those constructs the duocalins, either with **bispecific** or with bivalent target recognition properties, should provide useful reagents for various purposes in biotechnology.

L20 ANSWER 13 OF 15 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1999:584355 The Genuine Article (R) Number: 220MJ. Isolation and

characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 **bispecific** scFv that triggers CD16-dependent tumor cytolysis. McCall A M; Adams G P; Amoroso A R; Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; Marks J D; Weiner L M (Reprint). Fox Chase Canc Ctr, 7701 Burholme Ave, Philadelphia, PA 19111 USA (Reprint); Fox Chase Canc Ctr, Philadelphia, PA 19111 USA; San Francisco Gen Hosp, Dept Anesthesia, San Francisco, CA 94110 USA. MOLECULAR IMMUNOLOGY (**MAY 1999**) Vol. 36, No. 7, pp. 433-445. ISSN: 0161-5890. Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB **Bispecific** antibody (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal antibody (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of antibody Fc domains with cellular Fc receptors has limited the doses of antibody (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the HER2/neu protooncogene product and the human Fc gamma RIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from single-chain Fv (scFv) molecules specific for HER2/neu and CD16. The human anti-CD16 scFv component, NM3E2, was: isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fe binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/neu scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as fusion partner for the creation of a **bispecific** scFv (bs-scFv). In the presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian cancer cells overexpressing HER2/neu. Biodistribution studies performed in SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of I-125-labeled C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the **fusion protein.** This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5 x NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins with potential clinical applications as a result of its human origin, lack of an Fe domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting.

L20 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

2003:435072 Document No. 139:21017 Prostate-associated protease HUPAP, cDNA and antibodies for prognosis, diagnosis and treatment of prostate cancer. Spancake, Kimberly M.; Bandman, Olga; Lal, Preeti G. (Incyte Genomics, Inc., USA). U.S. Pat. Appl. Publ. US 2003103981 A1 20030605, 42 pp., Cont.-in-part of U.S. Ser. No. 988,975. (English). CODEN: USXXCO. APPLICATION: US 2002-235699 20020904. PRIORITY: US 1997-807151 19970227; US 2000-478957 20000107; US 2001-988975 20011119.

AB The invention provides a cDNA which encodes a human prostate-associated protease, or kallikrein designated as HUPAP, differentially expressed in prostate cancer. It also provides for the use of the cDNA, fragments, complements, and variants thereof and of the encoded protein, portions thereof and antibodies thereto for diagnosis and treatment of prostate cancer. The invention addnl. provides expression vectors and host cells for the production of the protein and a transgenic model system.

L20 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

1993:647978 Document No. 119:247978 Monomeric and dimeric antibody-fragment **fusion proteins** and their manufacture with recombinant cells. Plueckthun, Andreas; Pack, Peter (Merck Patent G.m.b.H., Germany).

PCT Int. Appl. WO 9315210 A1 **19930805**, 44 pp. DESIGNATED STATES: RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-EP82 19930115. PRIORITY: EP 1992-101069 19920123.

AB Single-chain Fv fragments fused (optionally through a linker peptide) to a peptide which can dimerize with another peptide by noncovalent interactions, and dimeric **fusion proteins** formed by this interaction are described. These monomeric and dimeric **fusion proteins** can be prepared by expression of chimeric genes in a suitable host cell, e.g. E. coli. Thus, a vector containing genes for an scFv-linker-fos protein leucine zipper peptide and an scFv-linker-jun protein leucine zipper expressed from a single promoter was prepared E. coli containing this vector produced a **bispecific** miniantibody which was purified by **affinity** chromatog. and which was characterized with respect to specificity and **affinity** of **ligand** binding.

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L25 ANSWER 1 OF 5 MEDLINE on STN
2001124847. PubMed ID: 11197965. Two-step targeting of xenografted colon carcinoma using a **bispecific antibody** and 188Re-labeled bivalent hapten: **biodistribution** and dosimetry studies. Gestin J F; Loussouarn A; Bardies M; Gautherot E; Gruaz-Guyon A; Sai-Maurel C; Barbet J; Curtet C; Chatal J F; Faivre-Chauvet A. (Institut de Biologie, Institut National de la Sante et de la Recherche Medicale, Nantes, France.) Journal of nuclear medicine : official publication, Society of Nuclear Medicine, **(2001 Jan)** Vol. 42, No. 1, pp. 146-53. Journal code: 0217410. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB Radioimmunotherapy (RIT) is currently being considered for the treatment of solid tumors. Although results have been encouraging for pretargeted 131I RIT with the affinity enhancement system (AES), the radionuclide used is not optimal because of its long half-life, strong gamma emission, poor specific activity, and low beta particle energy. 188Re, though unsuitable for direct antibody labeling, could be used with the AES two-step targeting technique. The purpose of this study was to compare the distribution and dosimetry of a bivalent hapten labeled with 188Re or 125I. For dosimetry calculations and **biodistribution** data, 125I was substituted for 131I. METHODS: After preliminary injection of a bispecific anticarcinoembryonic antigen (CEA) or antihapten antibody (Bs-mAb F6-679), AG 8.1 or AG 8.0 hapten radiolabeled with 188Re or 125I was injected into a nude mouse model grafted subcutaneously with a human colon carcinoma cell line (LS-174-T) expressing CEA. A dosimetry study was performed for each animal from the concentration of radioactivity in tumor and **different** tissues. RESULTS: Radiolabeling of AG 8.1 with 125I afforded a 40% yield with a specific activity of 11.1 MBq/nmol

after purification. Radiolabeling of AG 8.0 with 188Re afforded a 72% yield with a specific activity of 31.82 MBq/nmol. In all experiments, the percentage of tumor uptake of 125I-AG 8.1 was always significantly greater than that of 188Re-AG 8.0. The corresponding tumor-to-tissue ratios reflected uptake values. The least favorable tumor-to-normal tissue ratios in the dosimetry study were 8.1 and 8.5 for 131I (tumor-to-blood ratio and tumor-to-kidney ratio, respectively) and 2.3 for 188Re (tumor-to-intestine ratio). CONCLUSION: This study indicates that 188Re can be used for radiolabeling of hapten in two-step radioimmunotherapy protocols with the AES technique. 188Re has a greater range than 131I, which should allow the treatment of solid tumors around 1 cm in diameter. Although the method used for hapten radiolabeling did not provide optimal tumor uptake, the use of a bifunctional chelating agent associated with AG 8.1 should solve this problem.

L25 ANSWER 2 OF 5 MEDLINE on STN

96014015. PubMed ID: 7571036. Feasibility of radioimmunoguided surgery of colorectal carcinoma using indium 111 CEA specific antibody and simulation with a phantom using 2 steps targetting with **bispecific antibody.** Hamy A; Curtet C; Paineau J; Chatal J F; Visset J. (Clinique Chirurgicale, Chu Nantes, France.) Tumori, **(1995 May-Jun)** Vol. 81, No. 3 Suppl, pp. 103-6. Journal code: 0111356. ISSN: 0300-8916. Pub. country: Italy. Language: English.

AB The study was undertaken to define the potential use of radiolabelled (Indium 111 or Technetium 99 m) carcinoembryonic antigen specific antibody (CEA f(ab')2) for the radioimmunodetection of colorectal cancer using an intraoperative hand-held gamma probe. A clinical study performed with ten patients showed that tumor with good uptake of CEA specific antibody could be detected with sufficient contrast only in two patients. Results of a **biodistribution** study performed with tumor fragment and normal tissue countings in a gamma counter showed high tumor uptake in five patients. There was no correlation between tumor uptake and the count rates measured intraoperatively. To increase the signal/background of the gamma probe, a simulation study with a peritoneal cavity phantom was performed. We determined the efficiency of a two steps targetting method compared to the direct method. We simulated **different** tumor sizes with plexiglas balls (0.5, 1, 2, 5 ml) and tested two scintillators (NaI, BgO). Experiments were performed with 111 In and 99 m Tc. The two steps targetting method was better than direct method. The results of simulation with direct method radiolabelled with 111 in confirmed our clinical study: no efficiency of a gamma probe for the surgeon to detect a tumor. However the two steps targetting method (indirect labelling method) was very encouraging to detect tumors (size 1 and 2 ml) and definitively convincing with 99 m Tc.

L25 ANSWER 3 OF 5 MEDLINE on STN

95103511. PubMed ID: 7805020. Multistep tumor targeting in nude mice using **bispecific antibodies** and a gallium chelate suitable for immunoscintigraphy with positron emission tomography. Schuhmacher J; Klivenyi G; Matys R; Stadler M; Regiert T; Hauser H; Doll J; Maier-Borst W; Zoller M. (Department of Diagnostic and Therapeutic Radiology (FS5), German Cancer Research Center, Heidelberg.) Cancer research, **(1995 Jan 1)** Vol. 55, No. 1, pp. 115-23. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB To improve tumor:tissue ratios in immunoscintigraphy, a three-step targeting method has been developed. The reagents used were (a) a radioactive, low molecular weight chelate prepared from ionic gallium and a phenolic polyaminocarboxylic acid, which can be labeled either with the single-photon emitter 67Ga or with the short-lived positron emitter 68Ga (t1/2 = 68 min); (b) a bispecific monoclonal antibody (bs-mAb) synthesized from the F(ab)2 fragment of the 1.1ASML antibody specific for the glycoprotein CD44v associated with a rat pancreas carcinoma cell line and the F(ab') fragment of an antibody specific for the gallium chelate; and (c) the nonradioactive gallium chelate covalently coupled to transferrin, which served as a high molecular weight blocker to prevent binding of the

radioactive gallium chelate to bs-mAbs in the circulation. Targeting experiments in tumor-bearing nude mice with **different** doses of bs-mAbs, blocker, and 67Ga chelate were adjusted to maximize tumor to tissue contrasts and tumor uptake. Compared with the **biodistribution** of the 131I-labeled, native 1.1ASML antibody 24 h postinjection, a schedule using 100 pmol bs-mab 24 h later 100 pmol blocker, 15 min later 16 pmol 67Ga chelate, 1 h later examination, increased tumor:blood and tumor: liver ratios by a factor of 5 while keeping the localization of radioactivity in the tumor constant (10.1% injected dose/g). High-contrast images using either 67Ga or 68Ga were obtained within 1 h. The targeting method described enables the use of the short-lived positron emitter 68Ga and thus allows the combination of an improved immunoscintigraphy and positron emission tomography.

L25 ANSWER 4 OF 5 EMBASE

1998348890 EMBASE Gallium-68 chelate imaging of human colon carcinoma xenografts pretargeted with bispecific anti-CD44(V6)/anti-gallium chelate antibodies. Klivenyi G.; Schuhmacher J.; Patzelt E.; Hauser H.; Matys R.; Moock M.; Regiert T.; Maier-Borst W.. Dr. J. Schuhmacher, Dept. of Diagn. and Therap. Radiol., German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Journal of Nuclear Medicine Vol. 39, No. 10, pp. 1769-1776 **Oct 1998.**
Refs: 36.
ISSN: 0161-5505. CODEN: JNMEAQ
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 19981112. Last Updated on STN: 19981112

AB Recently, we demonstrated the feasibility of combining improved tumor-to-tissue contrasts and PET imaging for immunoscintigraphic tumor localization using a multistep targeting technique that consists of the administration of an antitumor/antihapten bispecific monoclonal antibody (BS- MAb), a blocker to saturate the antihapten binding sites of the BS-MAb that are still present in the circulation, and a low molecular weight Ga chelate, labeled with positron emitter (68)Ga, serving as the hapten. Due to this technique, the **biodistribution** of the radiolabeled hapten is governed mainly by the binding characteristics of both the antitumor and the antihapten part of the BS-MAb. For a future clinical implementation of the method, we investigated MAb VFF18, which is reactive with the adhesion molecule CD44(v6), a tumor-associated antigen, and up-regulated in colon, squamous cell and pancreas carcinoma, and two anti-Ga chelate MAbs, which are highly selective for only one of the two enantiomers (optical isomers) of the inherently racemic Ga chelate. Methods: From the VFF18 MAb and the anti-Ga chelate MAbs, two BS-MAbs containing the same antitumor parts, but **different** antihapten parts, were prepared and tested for multistep targeting in human colon carcinoma-bearing nude mice. Results: Despite identical **biodistributions** of both BS-MAbs and their very similar affinities for the corresponding Ga chelate enantiomers, tumor uptake of the two enantiomers 1 hr postinjection was significantly **different** [8.7 ± 1.9% versus 5.8% ± 1.6% of the injected dose/g (%ID/g)], with tumor-to-blood ratios being higher for the BS-MAb showing the lower tumor uptake (7.6 ± 1.6 versus 4.7 ± 0.6). From data obtained with each BS-MAb, a similar initial tumor binding of 15.5%ID/g, but **different** in vivo half-lives of the corresponding BS-MAb-enantiomer immune complexes, could be estimated. Pretargeting with a mixture of both BS-MAbs followed by the administration of the racemic Ga chelate resulted in the lowest tumor uptake (3.9% ± 1.5%ID/g). PET imaging of nude mice with the enantiomeric, as well as with the racemic, (68)Ga chelate demonstrated a clear delineation of tumors against blood pool background. Conclusion: Multistep immunoscintigraphy with BS-MAbs markedly increases tumor-to-tissue ratios in nude mice and enables PET imaging. Using a BS-MAb containing MAb VFF18, a more sensitive localization of CD44(v6)-positive tumors in patients should also be obtained.

L25 ANSWER 5 OF 5 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1995:821726 The Genuine Article (R) Number: TH916. DEVELOPMENT AND CHARACTERIZATION OF ANTIRENAL CELL-CARCINOMA X ANTICHELATE BISPECIFIC MONOCLONAL-ANTIBODIES FOR 2-PHASE TARGETING OF RENAL-CELL CARCINOMA. KRANENBORG M H G C (Reprint); BOERMAN O C; OOSTERWIJKWAKKA J C; DEWEIJERT M C A; CORSTENS F H M; OOSTERWIJK E. UNIV NIJMEGEN HOSP, DEPT NUCL MED, POB 9100, 6500 HB NIJMEGEN, NETHERLANDS (Reprint); UNIV NIJMEGEN HOSP, DEPT UROL, 6500 HB NIJMEGEN, NETHERLANDS. CANCER RESEARCH (**1 DEC 1995**) Vol. 55, No. 23, Supp. [S], pp. S5864-S5867. ISSN: 0008-5472. Publisher: AMER ASSOC CANCER RESEARCH, PUBLIC LEDGER BLDG, SUITE 816, 150 S. INDEPENDENCE MALL W., PHILADELPHIA, PA 19106. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB To test a two-step approach for radioimmunotargeting of renal cell cancer, quadroma cells secreting antichelate x anti-renal cell carcinoma **bispecific antibodies** were obtained by somatic cell fusion, Five monoclonal antibodies against the chelate 1,4,7-triaxaheptane-N,N',N''-pentaacetic acid (DTPA) were produced and characterized, Competitive binding assays indicated that the anti-DTPA antibodies reacted with DTPA chelated with indium, yttrium, chromium, iron, or zinc, The affinity constants of the anti;DTPA antibodies for In-111-DTPA ranged from 0.19 to 0.23 nM(-1). Using **different** chelates, a remarkable chelate specificity of the anti-DTPA antibodies was demonstrated, The chelates recognized by the antibodies DTIn1, DTIn2, and DTIn4 share a N(N'')-diacetic acid group, whereas the chelates recognized by DTIn3 share a N'-acetic acid group, suggesting the presence of **different** essential structures within the DTPA molecule that determine the reactivity of the antibodies.

Five anti-DTPA antibody-producing hybridomas were used for somatic cell fusion with hybridoma G250 directed against renal cell carcinoma, resulting in three **bispecific antibody**-producing quadroma cell lines, The bispecific monoclonal antibodies were purified from ascites fluid using protein A affinity chromatography followed by hydroxylapatite chromatography and/or cation exchange chromatography, Of the total IgG amount present in the ascites fluid, 10-15% represented the **bispecific antibodies**, These **bispecific antibodies** will allow testing and optimization of a two-step approach for radioimmunotargeting of chelated radionuclides.

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L26 18 L22 AND CANCER CELL

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L28 ANSWER 1 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2007:662432 The Genuine Article (R) Number: 168PU. Radiolabeling and targeting of lipidic nanocapsules for applications in radioimmunotherapy. Jestin, E.; Mougin-Degraef, M.; Faivre-Chauvet, A.; Remaud-Le Saec, P.; Hindre, F.; Benoit, J. P.; Chatal, J. F.; Barbet, J.; Gestin, J. F. (Reprint). Univ Nantes, INSERM, Dept Res Cancerol, U 601, 9 Quai Moncousu, F-44093 Nantes, France (Reprint); Univ Nantes, INSERM, Dept Res Cancerol, U 601, F-44093 Nantes, France; Univ Nantes, INSERM, U 646, F-44093 Nantes, France. jfgestin@nantes.inserm.fr. QUARTERLY JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING (MAR 2007) Vol. 51, No. 1, pp. 51-60. ISSN: 1824-4661. Publisher: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Aim. Radioimmunotherapy is limited in some cases by the low radioactive doses delivered to tumor cells by antibodies or pretargeted haptens. In order to increase this dose, lipidic nanocapsules (LNC) with a hydrophobic core are proposed as radionuclide vectors that could be targeted to **cancer cells** by a bispecific anti-tumor X anti-hapten antibody after incorporation of different haptens in the nanocapsule membrane.

Methods. To bind different radionuclides to the nanocapsules, several bifunctional chelating agents (BCA) were used to form stable complexes with the radionuclides. Some of them are hydrophilic for INC shell while others are lipophilic to radiolabel the core. Poly(ethylene glycols) (PEG) were used to increase the residence time in blood. Since PEG can modify haptens recognition by the **bispecific antibody** and radiolabeling efficiency, haptens, BCA or Bolton-Hunter reagent (BH) were attached to the PEG extremity to optimize accessibility Specific constructs (DSPE-PEG-haptens, DSPE-PEG-BCA, and DSPE-PEG-BH) were synthesized to develop these new radiolabeled vector formulations. Large amounts of PEG have been introduced by a postinsertion method without important change in nanocapsule size and properties. The nanocapsule core was radiolabeled with a lipophilic [Tc-99m]SSS complex.

Results. Serum stability studies showed that this Tc-99m-labeling method was efficient for at least 20 h. Concerning the nanocapsule surface, several methods have been performed for In-111-labeling by using DSPEPEG-DTPA and for I-125-labeling with DSPE-PEG-BH.

Conclusions. The nanocapsules labeling feasibility with a variety of radionuclides and their stability were demonstrated in this paper.

L28 ANSWER 2 OF 6 EMBASE

2005456491 EMBASE Pretargeting of carcinoembryonic antigen - Expressing tumors with a biologically produced bispecific anticarcinoembryonic antigen x anti-indium-labeled diethylenetriaminepentaacetic acid antibody.

Van Schaijk F.G.; Oosterwijk E.; Soede A.C.; Broekema M.; Frielink C.; McBride W.J.; Goldenberg D.M.; Corstens F.H.M.; Boerman O.C.. F.G. Van Schaijk, Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, Netherlands. F.vanSchaijk@hetnet.nl. Clinical Cancer Research Vol. 11, No. 19 II, pp. 7130s-7136s 1 Oct 2005.
Refs: 30.
ISSN: 1078-0432. CODEN: CCREF4
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 20051110. Last Updated on STN: 20051110

AB Purpose: The aim of these studies was to develop a pretargeting strategy for CEA-expressing cancers using biologically produced bispecific monoclonal antibodies (bsMAb). The bsMAbs used in this system have affinity for the carcinoembryonic antigen on the one hand, and for indium-labeled diethylenetriaminepentaacetic acid (DTPA), on the other. Experimental Design: Stable quadroma clones producing bsMAb MN-14xDTIn-1 were isolated. LS174T tumor - bearing mice were injected with 1 to 100 μg of bsMAb followed by 1 to 60 ng of an (111)In-labeled bivalent peptide [Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA) -NH(2)]. Mice were killed at 24 hours postinjection and the **biodistribution** of the radiolabel was determined. The **biodistribution** of diDTPA labeled with four different radionuclides ((111)In, (99m)Tc, nonresidualizing (125)I, and residualizing (125)I) was determined at various time points postinjection following pretargeting of LS174T tumors with bsMAb MN-14xDTIn-1. Results: Optimal tumor targeting was observed when tumors were pretargeted with 10 μg of bsMAb MN-14xDTIn-1 and when 6 ng of a radiolabeled peptide was given 72 hours later. The uptake of the four radiolabels in LS174T tumors at 4 hours postinjection was similar. However, at later time points, the (111)In-label and residualizing (125)I-label were better retained in the tumor than the nonresidualizing (126)I label. Although the absolute uptake in the tumor (in terms of percentage of injected dose per gram of

tissue) was 5-fold lower than the uptake obtained with directly labeled MN-14, the pretargeting strategy revealed much higher tumor-to-blood ratios due to the rapid clearance of the radiolabel from the circulation as compared with (111)In-MN-14 (445 ± 90 and 5.3 ± 1.1, respectively, at 72 hours postinjection). Conclusions: Effective targeting of carcinoembryonic antigen-expressing tumors was achieved with a newly produced **bispecific antibody.** The (111)In-labeled L-amino acid peptide and (125)I-D-amino acid peptide were better retained in the tumor than the (99m)Tc- and (125)I-L- amino acid peptide. Very high tumor-to-blood ratios were obtained due to rapid background clearance.

L28 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2001:502429 Document No.: PREV200100502429. Targeting properties of an anti-CD16/anti-CD30 **bispecific antibody** in an in vivo system. Renner, Christoph [Reprint author]; Stehle, Ingo; Lee, Fook Thean; Hall, Cathrine; Catimel, Bruno; Nice, Edouard C.; Mountain, Angela; Rigopoulos, Angela; Brechbiel, Martin W.; Pfreundschuh, Michael; Scott, Andrew M.. Medical Department I, Saarland University, Kirrberger Strasse, 66424, Homburg/Saar, Homburg, Germany. Cancer Immunology Immunotherapy, (April, 2001) Vol. 50, No. 2, pp. 102-108. print.
CODEN: CIIMDN. ISSN: 0340-7004. Language: English.

AB **Bispecific antibodies** are currently being used in clinical trials in increasing numbers in the areas of breast cancer, prostate cancer, non-Hodgkin's lymphoma and Hodgkin's lymphoma. We have previously performed two clinical trials in patients with Hodgkin's disease with an anti-CD30/anti-CD16 **bispecific antibody** and demonstrated a 30% response rate in a cohort of patients otherwise resistant to standard therapeutic modalities. However, no surrogate marker could be de-fined in these trials indicative of optimal antibody dosing/scheduling or predictive for favorable response. In order to evaluate accurately the potential **biodistribution** properties of **bispecific antibody** in patients, we have performed a detailed analysis of the binding properties and animal model in vivo characteristics of these constructs. For this purpose, the parental antibodies (anti-CD30 and anti-CD16) and the **bispecific antibody** (anti-CD30/anti-CD16) were radiolabeled with either 125I or 111In. Antibody integrity and binding properties after labeling were confirmed by Scatchard plot and Lindmo analysis. 111In-labeled antibodies revealed superior targeting properties in a standard SCID mouse tumor model. Both the bivalent parental anti-CD30 monoclonal antibody and the monovalent anti-CD30/anti-CD16 **bispecific antibody** showed excellent uptake in CD30+ tumors which did not differ significantly between the two (maximum uptake 16.5%+-4.2% vs. 18.4%+-3.8% injected dose/gram tissue). The equivalent targeting properties of the **bispecific antibody** compared with the parental anti-CD30 antibody encourages the further clinical development of this **bispecific antibody**, and might help to explain the clinical responses seen with this antibody so far in patients suffering from Hodgkin's disease.

L28 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 1
1999332030. PubMed ID: 10405144. Two-step targeting and dosimetry for small cell lung cancer xenograft with anti-NCAM/antihistamine **bispecific antibody** and radioiodinated bivalent hapten.
Hosono M; Hosono M N; Kraeber-Bodere F; Devys A; Thedrez P; Faivre-Chauvet A; Gautherot E; Barbet J; Chatal J F. (Department of Radiology, Saitama Medical Center, Saitama Medical School, Kawagoe, Japan.) Journal of nuclear medicine : official publication, Society of Nuclear Medicine, (1999 Jul) Vol. 40, No. 7, pp. 1216-21. Journal code: 0217410. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB The "affinity enhancement system," a two-step targeting technique using **bispecific antibody** and radiolabeled bivalent hapten, has been reported to be useful for carcinoembryonic antigen-expressing tumors. The purpose of this study was to evaluate the efficacy of this

method for targeting human small cell lung cancer using an antineural cell adhesion molecule antibody. METHODS: Antineural cell adhesion molecule/antihistamine **bispecific antibody** NK1NBL1-679 was prepared by coupling an equimolecular quantity of a Fab' fragment of NK1NBL1 to a Fab fragment of antihistamine 679. Athymic mice inoculated with NCI-H69 small cell lung **cancer cells** expressing neural cell adhesion molecule were administered **bispecific antibody** and then 48 h later 125I-labeled bivalent histamine hapten. 125I-labeled intact NK1NBL1 was injected into other groups of mice. **Biodistributions** were examined as a function of time. RESULTS: In mice of the two-step targeting, tumor uptake was 2.5 +/- 0.2, 3.2 +/- 0.4, 6.4 +/- 2.0, 7.2 +/- 2.7, 6.1 +/- 2.1 and 2.2 +/- 0.4 %ID/g at 5, 30 min, 5, 24, 48 and 96 h, and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 1.4 +/- 1.1, 10.8 +/- 13.2 and 4.6 +/- 4.7, respectively, at 5 h, whereas 125I-labeled NK1NBL1 showed a tumor uptake of 5.7 +/- 0.4 %ID/g and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of 0.3 +/- 0.1, 1.1 +/- 0.2 and 0.9 +/- 0.1, respectively, at 5 h. These results were confirmed by autoradiographic studies, which demonstrated clear tumor-to-normal tissue contrast. Dosimetry showed that the affinity enhancement system could enhance the therapeutic potential of the antineural cell adhesion molecule antibody NK1NBL1. CONCLUSION: This two-step targeting method seems promising for the diagnosis and therapy of small cell lung cancer.

L28 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 2

1999376336. PubMed ID: 10449096. Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis. McCall A M; Adams G P; Amoroso A R; Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; Marks J D; Weiner L M. (Fox Chase Cancer Center, Philadelphia, PA 19111, USA.) Molecular immunology, (1999 May) Vol. 36, No. 7, pp. 433-45. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Bispecific antibody** (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal antibody (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of antibody Fc domains with cellular Fc receptors has limited the doses of antibody (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the HER2/neu protooncogene product and the human FcgammaRIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from single-chain Fv (scFv) molecules specific for HER2/neu and CD16. The human anti-CD16 scFv component, NM3E2, was isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fc binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/neu scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as fusion partner for the creation of a bispecific scFv (bs-scFv). In the presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian **cancer cells** overexpressing HER2/neu. **Biodistribution** studies performed in SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of 125I-labeled C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the fusion protein. This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5 x NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins with potential clinical applications as a result of its human origin, lack of an Fc domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting.

L28 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 3
1998415602. PubMed ID: 9744353. **Biodistribution** and dosimetric study in medullary thyroid cancer xenograft using **bispecific antibody** and iodine-125-labeled bivalent hapten. Hosono M; Hosono M N; Kraeber-Bodere F; Devys A; Thedrez P; Fiche M; Gautherot E; Barbet J; Chatal J F. (Saitama Medical Center, Saitama Medical School, Kawagoe, Japan.) Journal of nuclear medicine : official publication, Society of Nuclear Medicine, (1998 Sep) Vol. 39, No. 9, pp. 1608-13. Journal code: 0217410. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB The purpose of this study was to evaluate **biodistributions** and absorbed doses of anti-carcinoembryonic antigen (CEA)/anti-diethylenetriamine pentaacetic acid (DTPA)-indium (anti-DTPA-In) bispecific monoclonal antibody (BsMAb) F6-734 and 125I-labeled DTPA-indium dimer hapten (125I-di-DTPA-In hapten) in athymic mice xenografted with human medullary thyroid cancer. METHODS: Bispecific monoclonal antibodies F6-679 (anti-CEA/antihistamine) and G7A5-734 (antimelanoma/anti-di-DTPA-In) were used as irrelevant BsMAbs. Athymic mice inoculated with TT medullary thyroid **cancer cells** expressing CEA were administered BsMAbs F6-734, F6-679 or G7A5-734 and then, 48 hr later, 125I-di-DTPA-In hapten. Iodine-125-labeled F6 F(ab')2 fragment was injected into other groups of mice. **Biodistributions** were examined at 30 min and 5, 24, 48 and 96 hr after injection of 125I-di-DTPA-In hapten or 125I-labeled F6 F(ab')2. RESULTS: In mice injected with BsMAb F6-734 and 125I-di-DTPA-In hapten, tumor uptake was 9.1%+/-2.1%, 8.7%+/-3.5%, 8.0%+/-2.3%, 5.1%+/-0.9% and 3.5%+/-1.5% of the injected dose/g at 30 min and 5, 24, 48 and 96 hr, and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 37.0+/-12.5, 32.3+/-10.9 and 10.4+/-2.7 at 24 hr. Iodine-125-F6 F(ab')2 fragment showed a tumor uptake of 7.39% injected dose/g and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of 1.8+/-0.6, 7.3+/-2.9 and 3.6+/-1.6 at 24 hr. In mice injected with F6-679 or G7A5-734, tumor uptake and tumor-to-normal tissue ratios were much lower than in the mice injected with F6-734. These results were confirmed by autoradiographic studies that demonstrated clear tumor-to-normal tissue contrast. CONCLUSION: This two-step targeting method seems very potent for the diagnosis and therapy of human medullary thyroid cancer and other CEA-producing tumors because it combines high tumor uptake and low normal tissue background.

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L30           4 L29 AND TARGETED LIGANDS

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L31 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:570440 Document No. 143:95829 **Targeted ligands**: antibody-based enhancement of conventional therapy. **Herman, William** (Can.). U.S. Pat. Appl. Publ. US 2005142539 A1 20050630, 86 pp., Cont.-in-part of Appl. No. PCT/CA03/00044. (English). CODEN: USXXCO. APPLICATION: US 2004-943918 20040920. PRIORITY: CA 2002-2368708 20020114; CA 2002-2397169 20020813; CA 2002-2402930 20020919; WO 2003-CA44 20030114; US 2003-504283P 20030919.

AB The author discloses multispecific ligands, for example a heterofunctional ligand, comprising at least first and second binding moieties which have cooperating functional affinities. As an illustration, the multispecific ligand can comprise a bispecific antibody to a cell-specific marker and a second portion which binds to a cell-surface receptor. In a specific

example, the multispecific ligand is a bispecific single-chain antibody directed to CEA and P-glycoprotein. Functional studies demonstrate that the bispecific antibody increases the drug sensitivity of tumor cells expressing both antibody targets.

L31 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:23253 Document No. 142:296677 Design/selection of ligands or antibodies specific to circulating antigen or cell for treating cancer, infection and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2385690 C 20030924, 49 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2385690 20020324.

AB The present invention relates to fluid dynamically improved ligands including immunoligands, immunoconjugates, immunotoxins and bispecific antibodies; and methods of making them. The fluid **targeted ligands** comprise a targeting ligand such as VH domain, VL domain, growth factor, cytokine, etc., operatively linked to a functional moiety such as toxin, cytokine, chemokine, growth factor or labeling agent. The ligands have improved affinity toward circulating targets (e.g. in blood) and are prepared by combinatorial method. The ligands or antibodies are useful for immunodiagnosis and immunotherapy of cancer, metastasis, infection, immune disease, autoimmune disease, and graft/transplant rejection.

L31 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:64214 Document No. 142:296678 Design of fluid dynamic-optimized targeting ligands or antibodies for diagnosis and treatment of cancer, infection, immune disease and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2379586 A1 20031010, 65 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2379586 20020410.

AB The present invention relates to improved ligands including immunoligands, immunoconjugates, immunotoxins and bispecific antibodies, and methods of making them. The fluid **targeted ligands** comprise a targeting ligand such as VH domain, VL domain, growth factor, cytokine, Fc etc. operatively linked to a functional moiety such as toxin, cytokine, chemokine, growth factor or labeling agent. The ligands have improved affinity and are prepared by combinatorial method. The ligands or antibodies are useful for immunodiagnosis and immunotherapy of cancer, metastasis, infection, immune disease, autoimmune disease, and graft/transplant rejection.

L31 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:9133 Document No. 142:303593 **Targeted ligands** for improvements in cancer therapy. **Herman, William** (Can.). Can. Pat. Appl. CA 2376356 A1 20030920, 22 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2376356 20020320.

AB The disclosed invention relates to improvements in cancer therapy and targeted cancer therapy. Solid tumors are often refractory to therapy with macromols. (e.g. antibodies, growth factors, colony-stimulating factors, cytokines, chemokines, immunofusions, immunoconjugates), at least partly due to the inability of these mols. to penetrate tight spaces between the tumor cells. The disclosed invention thus relates to a composition adapted to preferentially target tumors, said composition comprising a solute or preferably a liquid which increases the intracellular osmolality of cancer cells within the tumor so as to cause tumor rupture. In one embodiment the invention is directed to a tumor-targeted composition which comprises a vehicle, e.g. a carrier such as vesicle or microparticle/nanoparticle which preferentially enters or remains in tumor tissues. The carrier may be a liposome or an immunoliposome. The invention contemplates that tumor-targeted ion-containing liposomes/immunoliposomes may be used with ≥1 of the following: an antibody (avidin/streptavidin-conjugated); an immunoliposome comprising a tonicity-modifying agent (e.g. ions), which is targeted with suitable affinity to an angiogenic marker which facilitates/promotes liposome internalization; an immunocytokine (tumor necrosis factor, interleukin-12, interleukin-2) and the targeting moiety is targeted with suitable affinity

to a tumor marker; relaxin; immunoconjugates with e.g. radionuclides or immunotoxins conjugated to radionuclides; and immunoenzymes adapted to ADEPT therapy.

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L34 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:570440 Document No. 143:95829 Targeted ligands: antibody-based enhancement of conventional therapy. **Herman, William** (Can.). U.S. Pat. Appl. Publ. US 2005142539 A1 20050630, 86 pp., Cont.-in-part of Appl. No. PCT/CA03/00044. (English). CODEN: USXXCO. APPLICATION: US 2004-943918 20040920. PRIORITY: CA 2002-2368708 20020114; CA 2002-2397169 20020813; CA 2002-2402930 20020919; WO 2003-CA44 20030114; US 2003-504283P 20030919.
AB The author discloses multispecific ligands, for example a heterofunctional ligand, comprising at least first and second binding moieties which have cooperating functional affinities. As an illustration, the multispecific ligand can comprise a **bispecific** antibody to a cell-specific marker and a second portion which binds to a cell-surface receptor. In a specific example, the multispecific ligand is a **bispecific** single-chain antibody directed to CEA and P-glycoprotein. Functional studies demonstrate that the **bispecific** antibody increases the drug sensitivity of tumor cells expressing both antibody targets.

L34 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:479747 Document No. 143:6295 Multispecific antibodies specific to cell surface antigen and/or receptor for immunotherapy of autoimmune disease, transplant rejection, infection and cancer. **Herman, William** (Can.). Can. Pat. Appl. CA 2441653 A1 20050319, 166 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2003-2441653 20030919.
AB The invention contemplates a composition containing a multispecific ligand containing at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand. The first ligand has a first biodistribution. The second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand. The second ligand has a second biodistribution. The affinities of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favor of a selected location of one or both of the ligands. The first ligand is a cell surface marker or antigen associated with infectious or parasitic agents, diseased cells or disease-associated cells; and the second ligand is a CCR5, **CXCR4**, tyrosine kinase receptor, serine kinase receptor, Notch family receptor, decoy receptor, adhesion receptor, IL-8 receptor, CCR7, Fas receptor, etc.

L34 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2005:102407 Document No. 142:315222 multispecific ligands or antibodies for targeting and treating cancer, infection and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2414148 A1 20040630, 109 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2414148 20021230.
AB The invention contemplates a composition containing a multispecific ligand containing

at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand or cell surface marker associated with specific cell, infectious or parasitic agent, e.g. antigen, epitope, CD marker, CD4. The first ligand has a first biodistribution. The second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand, e.g CCR5, **CXCR4**, tyrosine kinase receptor, serine kinase receptor, G protein-coupled receptor, TNF receptor, NOTCH receptor, decoy receptor, adhesion receptor, IL-8 receptor, CCR7 receptor, etc. The second ligand has a second biodistribution. The affinity of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favor of a selected location of one or both of the ligands.

L34 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN
2003:551546 Document No. 139:83987 Multispecific constructs for targeted therapy. **Herman, William** (Can.). PCT Int. Appl. WO 2003057732 A2 20030717, 240 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-CA44 20030114. PRIORITY: CA 2002-2368708 20020114; WO 2002-CA317 20020311; CA 2002-2397169 20020813; CA 2002-2402930 20020919.
AB The author discloses multispecific agents containing at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand; the second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand. In one embodiment, the multispecific agent is a **bispecific** antibody.

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L35 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:570440 Document No. 143:95829 Targeted ligands: antibody-based enhancement of conventional therapy. **Herman, William** (Can.). U.S. Pat. Appl. Publ. US 2005142539 A1 20050630, 86 pp., Cont.-in-part of Appl. No. PCT/CA03/00044. (English). CODEN: USXXCO. APPLICATION: US 2004-943918 20040920. PRIORITY: CA 2002-2368708 20020114; CA 2002-2397169 20020813; CA 2002-2402930 20020919; WO 2003-CA44 20030114; US 2003-504283P 20030919.
AB The author discloses multispecific ligands, for example a heterofunctional ligand, comprising at least first and second binding moieties which have cooperating functional affinities. As an illustration, the multispecific ligand can comprise a **bispecific** antibody to a cell-specific marker and a second portion which binds to a cell-surface receptor. In a specific example, the multispecific ligand is a **bispecific** single-chain antibody directed to CEA and P-glycoprotein. Functional studies demonstrate that the **bispecific** antibody increases the drug sensitivity of tumor cells expressing both antibody targets.

L35 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:479747 Document No. 143:6295 Multispecific antibodies specific to cell surface antigen and/or receptor for immunotherapy of autoimmune disease, transplant rejection, infection and cancer. **Herman, William** (Can.). Can. Pat. Appl. CA 2441653 A1 20050319, 166 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2003-2441653 20030919.

AB The invention contemplates a composition containing a multispecific ligand containing at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand. The first ligand has a first biodistribution. The second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand. The second ligand has a second biodistribution. The affinities of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favor of a selected location of one or both of the ligands. The first ligand is a cell surface marker or antigen associated with infectious or parasitic agents, diseased cells or disease-associated cells; and the second ligand is a CCR5, CXCR4, tyrosine kinase receptor, serine kinase receptor, Notch family receptor, decoy receptor, adhesion receptor, IL-8 receptor, CCR7, Fas receptor, etc.

L35 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:102407 Document No. 142:315222 multispecific ligands or antibodies for targeting and treating cancer, infection and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2414148 A1 20040630, 109 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2414148 20021230.
AB The invention contemplates a composition containing a multispecific ligand containing at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand or cell surface marker associated with specific cell, infectious or parasitic agent, e.g. antigen, epitope, CD marker, CD4. The first ligand has a first biodistribution. The second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand, e.g CCR5, CXCR4, tyrosine kinase receptor, serine kinase receptor, G protein-coupled receptor, TNF receptor, NOTCH receptor, decoy receptor, adhesion receptor, IL-8 receptor, CCR7 receptor, etc. The second ligand has a second biodistribution. The affinity of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favor of a selected location of one or both of the ligands.

L35 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2003:551546 Document No. 139:83987 Multispecific constructs for targeted therapy. **Herman, William** (Can.). PCT Int. Appl. WO 2003057732 A2 20030717, 240 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-CA44 20030114. PRIORITY: CA 2002-2368708 20020114; WO 2002-CA317 20020311; CA 2002-2397169 20020813; CA 2002-2402930 20020919.
AB The author discloses multispecific agents containing at least a first ligand binding moiety and a second ligand binding moiety. The first ligand binding moiety specifically binds with a pre-selected first affinity to at least a first ligand; the second ligand binding moiety specifically binds with a pre-selected affinity to at least a second ligand. In one embodiment, the multispecific agent is a **bispecific** antibody.

L35 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:23253 Document No. 142:296677 Design/selection of ligands or antibodies specific to circulating antigen or cell for treating cancer, infection and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2385690 C 20030924, 49 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2385690 20020324.
AB The present invention relates to fluid dynamically improved ligands

including immunoligands, immunoconjugates, immunotoxins and **bispecific** antibodies; and methods of making them. The fluid targeted ligands comprise a targeting ligand such as VH domain, VL domain, growth factor, cytokine, etc., operatively linked to a functional moiety such as toxin, cytokine, chemokine, growth factor or labeling agent. The ligands have improved affinity toward circulating targets (e.g. in blood) and are prepared by combinatorial method. The ligands or antibodies are useful for immunodiagnosis and immunotherapy of cancer, metastasis, infection, immune disease, autoimmune disease, and graft/transplant rejection.

L35 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:64214 Document No. 142:296678 Design of fluid dynamic-optimized targeting ligands or antibodies for diagnosis and treatment of cancer, infection, immune disease and autoimmune disease. **Herman, William** (Can.). Can. Pat. Appl. CA 2379586 A1 20031010, 65 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2002-2379586 20020410.
AB The present invention relates to improved ligands including immunoligands, immunoconjugates, immunotoxins and **bispecific** antibodies, and methods of making them. The fluid targeted ligands comprise a targeting ligand such as VH domain, VL domain, growth factor, cytokine, Fc etc. operatively linked to a functional moiety such as toxin, cytokine, chemokine, growth factor or labeling agent. The ligands have improved affinity and are prepared by combinatorial method. The ligands or antibodies are useful for immunodiagnosis and immunotherapy of cancer, metastasis, infection, immune disease, autoimmune disease, and graft/transplant rejection.

L35 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:8172 Document No. 142:296676 Multifunctional ligands or antibodies for immunotherapy of immune disease, autoimmune disease, transplant rejection, infection and cancer. **Herman, William** (Can.). Can. Pat. Appl. CA 2365636 A1 20030605, 107 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2001-2365636 20011205.
AB The present invention relates to heterofunctional ligand comprising first and second moieties which have cooperating functional affinities as well as a functional ligand. For example, the ligand is a **bispecific** antibody having at least a first portion which binds to a lymphatic vessel-associated antigen or receptor and a second portion having at least one immune-affecting functionality related to e.g. antigen presentation, immune signalling, suppression of enhancement of immune tolerance or immunostimulation, or binding to a target mol. such as a cell surface antigen, receptor, etc.

L35 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2005:132263 Document No. 142:278735 Multifunctional ligands or antibodies for immunotherapy of autoimmune disease, transplant rejection, infection and cancer. **Herman, William** (Can.). Can. Pat. Appl. CA 2357529 A1 20030228, 122 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2001-2357529 20010830.
AB The present invention relates to heterofunctional ligand comprising first and second moieties which have cooperating functional affinities as well as a functional ligand. For example, the ligand is a **bispecific** antibody having at least a first portion which binds to a lymphatic vessel-associated antigen or receptor and a second portion having at least one immune-affecting functionality related to e.g. antigen presentation, immune signalling, suppression of enhancement of immune tolerance or immunostimulation, or binding to a target mol. such as a cell surface antigen, receptor, etc.

L35 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2004:1048638 Document No. 142:296675 Multifunctional ligand. **Herman, William** (Can.). Can. Pat. Appl. CA 2356413 A1 20030117, 18 pp. (English). CODEN: CPXXEB. APPLICATION: CA 2001-2356413 20010717.
AB The present invention facilitates development, therapeutic evaluation, and

delivery, particularly targeted delivery of mols. that exert therapeutic functions and particularly immune functions. In one aspect the invention is directed to heterofunctional ligand comprising a first moiety which binds to a first target ligand and a second moiety which binds to a second target ligand. The affinity or avidity (or both) of the first moiety and second moiety is selected to enable independent binding to the cognate targets. For example, in one embodiment the first moiety is divalent and the second moiety is monovalent. In another embodiment the first moiety is trivalent and the second moiety is monovalent. In a specific implementation, the heterofunctional ligand is an antibody.

L35 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2007 ACS on STN
2004:869162 Document No. 142:233296 Consensus peptide presenting entities, screening methods, and use for the treatment and diagnosis of tumors.. Maiti, Pradip K.; **Herman, William**; Dan, Michael D.; Kaplan, Howard A.; MacDonald, Glen C.; Entwistle, Jocelyn M.; Lewis, Keith E.; Fast, Darren G. (Novopharm Biotech Inc., Can.). Can. Pat. Appl. CA 2290722 A1 20010608, 155 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1999-2290722 19991208.

AB The invention provides antigen-binding-fragments specific for tumor cells and effective in treatment and/or diagnosing tumors. Methods of use are also provided as are methods for screening for addnl. such antigen-binding-fragments and the products obtained thereby.

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FULL ESTIMATED COST	223.97	224.18
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